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# Functional connectivity is preserved but reorganized across several anesthetic regimes

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## Abstract

Under anesthesia, systemic variables and CBF are modified. How does this alter the connectivity measures obtained with rs-fMRI? To tackle this question, we explored the effect of four different anesthetics on Long Evans and Wistar rats with multimodal recordings of rs-fMRI, systemic variables and CBF. After multimodal signal processing, we show that the blood-oxygen-level-dependent (BOLD) variations and functional connectivity (FC) evaluated at low frequencies (0.031 - 0.25 Hz) do not depend on systemic variables and are preserved across a large interval of baseline CBF values. Based on these findings, we found that most brain areas remain functionally active under any anesthetics, i.e. connected to at least one other brain area, as shown by the connectivity graphs. In addition, we quantified the influence of nodes by a measure of functional connectivity strength to show the specific areas targeted by anesthetics and compare correlation values of edges at different levels. These measures enable us to highlight the specific network alterations induced by anesthetics. Altogether, this suggests that changes in connectivity could be evaluated under anesthesia, routinely used in the control of neurological injury.

**Keywords:** anesthesia, cerebral blood flow (CBF), functional connectivity (FC), rat, resting state functional magnetic resonant imaging (rs-fMRI), systemic variables

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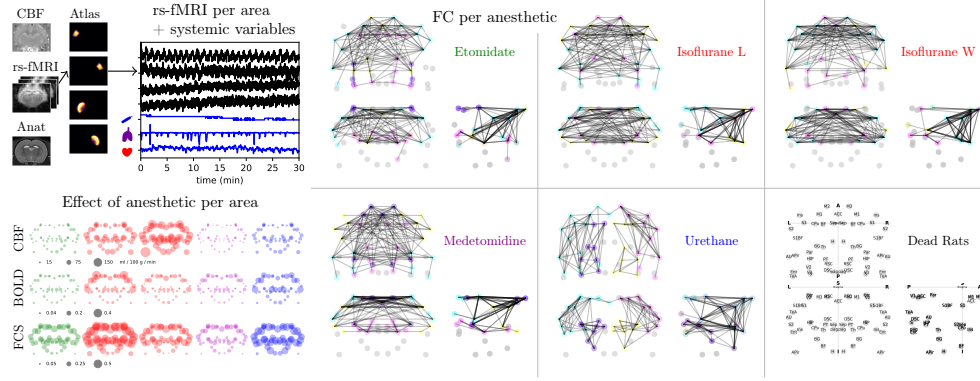
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## Graphical Abstract



## Highlights

- Different anesthetics lead to different systemic and brain alterations.
- BOLD variations and functional connectivity are affected by anesthesia.
- Systemic variables have little influence on functional connectivity.
- Baseline cerebral blood flow has no influence on functional connectivity.
- Anesthetics reshape distributions of connections.

## Abbreviations

BOLD (blood-oxygen-level dependent),  $\langle C \rangle$  (average correlation between signals of brain areas, or systemic signals), CBF (cerebral blood flow), FC (functional connectivity assessed by correlations between signals of brain areas in this study), FCS (functional connectivity strength, average correlation for one brain area), rs-fMRI (resting state functional magnetic resonant imaging), SV (systemic variables) such as HR (heart rate), RR (respiratory rate) and Temp (temperature).

# 1. Introduction

Resting state fMRI (rs-fMRI) is an elegant way to evaluate brain functional connectivity (FC) across species especially on Long Evans, Sprague Dawley or Wistar rats frequently used in the laboratory [1, 2, 3, 4, 5, 6]. While it is easy to ask a human subject to stay still, animals are either trained or anesthetized to limit motion artifacts [7, 2] (see also Appendix A). Training animals may however be too time consuming to be used in routine analysis and the stress from being restrained in a noisy environment could alter FC. The drawbacks of anesthesia are also well-known: it may change systemic variables (SV) such as temperature (Temp), heart rate (HR), respiration rate (RR) and at the brain level, it may alter neural activity, brain metabolism, baseline cerebral blood flow (CBF), and neurovascular coupling [8, 9, 10, 11]. A change in any of these variables may alter the FC mapped by rs-fMRI. This is why preclinical rs-fMRI data, obtained mostly under anesthesia, have been considered with some suspicion. This is related to two main questions: How can this apparent FC estimated from correlations between regional time series, reflect true brain connectivity? Is it possible, using standard methods, to evaluate whether brain connectivity is modified by the anesthetic? In order to map the impact of anesthesia on brain activity and metabolism, we want to evaluate potential biases on FC caused by confounding factors, SV and baseline CBF. Regarding SV, for a given data acquisition and processing protocol, changes in HR or RR may lead to change in the way the BOLD signal is contaminated by physiological noise [12, 13, 7, 14]. Anesthesia might change the frequency of microvascular oscillations, thus requiring an adaptation of the frequency band at which FC should be observed [15, 4, 16]; The effect of anesthesia on the regulation of Temp is also complex and results generally in an important cooling of the animal [17]. Baseline CBF is expected to alter the amplitude of the BOLD response induced by neural activity [18]. It has also been reported that changes in CBF may lead to changes in FC. Petrinovic et al. [19] acknowledge that an increase in baseline CBF could limit neurovascular reactivity and Jann et al. [20] observed positive correlations in human subjects between regional CBF and FC in all resting brain networks. Furthermore, Jonckers et al. [21] suggested that an increase in the variance of the BOLD signal could result in correlations that are less localized. A change in baseline CBF might thus modify the variance of the BOLD signal, the derived correlation coefficients, and thereby the apparent FC.

In this study, we explore large ranges of SV and baseline CBF using four different anesthetics and compare FC derived from long-run rs-fMRI recorded during 30 min with fast acquisition at 2 Hz. A processing pipeline to pick up the appropriate frequency bands to map FC is proposed. We show that FC is modified but preserved under anesthesia. Graphs of FC show different patterns and areas targeted by anesthetics.

## 2. Materials and methods

### 2.1. Animals

All experiments were approved by the local ethics committee and were performed in full compliance with the guidelines of the European community (EUVD 86/609/EEC) for the care and use of the laboratory animals, under permits from the French Ministry of Agriculture (number 380820 for EB and B3851610008 for experimental and animal care facilities). Experiments were conducted in the animal research facility of the Grenoble Institute of Neurosciences, a Specific Pathogen Free (SPF) housing facility, and comply with the ARRIVE guidelines [22]. Male rats were housed in enriched cages with 3 animals per cage and access to food and water ad libitum. Acquisitions dedicated to one anesthetic were conducted one after the other, with animals ascribed



Table 1: Systemic variables and baseline CBF.

	Eto-L	Iso-L	Iso-W	Med-L	Ure-L	Dead
N	7	6 (5)	7	7	7 (5)	4
W.	191 $\pm$ 7	254 $\pm$ 55	316 $\pm$ 86	295 $\pm$ 23	289 $\pm$ 29	333 $\pm$ 51
Temp.	36 $\pm$ 2	38 $\pm$ 0	37 $\pm$ 1	37 $\pm$ 0	38 $\pm$ 1	–
HR	343 $\pm$ 51	444 $\pm$ 26	350 $\pm$ 34	219 $\pm$ 16	383 $\pm$ 83	–
RR	67 $\pm$ 11	67 $\pm$ 11	70 $\pm$ 3	73 $\pm$ 25	108 $\pm$ 18	–
SpO2	99 $\pm$ 1	96 $\pm$ 5	99 $\pm$ 0	98 $\pm$ 1	75 $\pm$ 40	–
N	7	6	6	6	7	–
CBF	25 $\pm$ 6	67 $\pm$ 10	101 $\pm$ 27	19 $\pm$ 2	41 $\pm$ 9	–

Notations: N - number of rats, *italic indicates missing physiological measures for some individuals*; W. weight (g); Temp. - temperature ( $^{\circ}$ C); HR - heart rate in beats per minute (bpm), RR - respiration rate in cycles per minute (cpm); SpO2 - peripheral oxygen saturation (%) measured on the paw; CBF - baseline CBF (ml / 100 g / min)

to the group upon arrival order, with no other randomization. Once all anesthetics were tested, about half the data was collected. A second run of acquisitions was then performed to obtain the full dataset with a population target of at least 6 fMRI recordings per anesthetized group.

## 2.2. Experimental protocol

Four anesthetics are evaluated on Long Evans rats: Etomidate (Eto-L), Isoflurane (Iso-L), Medetomidine (Med-L) and Urethane (Ure-L), with standard dosages. After an induction of anesthesia with a gaseous mixture of isoflurane, air and oxygen, the anesthetics of the four groups are the following during rs-fMRI: - Isoflurane (Iso): the level of the isoflurane is set to 1 %. - Etomidate (Eto): continuous intravenous infusion at 0.5 mg / kg / min. - Medetomidine (Med): bolus then continuous infusion at 0.05 mg / kg / h. - Urethane (Ure): bolus of 1.25 g / kg injected intraperitoneally. To evaluate whether animal strain biased this comparison, Isoflurane was also administered to Wistar rats (Iso-W). A group of dead rats (Dead) was also created by sacrificing 4 animals. Overall, six groups were evaluated. The number of animals per group is given in Table 1. Each Animal (n=34) was included in only one anesthetic group and was recorded only one time, sacrificed if assigned to the Ure group or reused and sacrificed when randomly assigned to the Dead group (n=4 with 1 Med-L, 2 Ure-L and 1 Iso-W). In this last group, recordings begin one hour after sacrifice. Some recordings in CBF and SV were not available because of technical, measurements or artefacts problems and were excluded from the study (cf. Table 1).

During rs-fMRI sessions, systemic variables are monitored and recorded: from a pulse oximeter with a rat sensor clipped on the right posterior paw of the animal; a device measuring temperature with a rectal probe and respiration rate from a small pneumatic pillow sensor. Measurements from the two systems are sampled at 1 Hz. During the experiments a warm circulating water positioned below the animal is regulated to maintain the body temperature to a target level of 37  $^{\circ}$ C inside the MRI device. More technical and supplementary descriptions are given in Appendix B.

## 2.3. MRI Acquisitions at 9.4 T and data processing

CBF is mapped using pseudo continuous arterial spin labeling (pCASL) following inter-pulse phase optimization as proposed in [23] with a spatial resolution 0.47 by 0.47 by 1.00 mm, gap 0.1 mm, 9 slices. rs-fMRI was performed with a single shot echo-planar imaging with TR/TE

73 = 500/20 ms, the same spatial resolution as CBF, and 3600 repetitions (30 min). fMRI BOLD  
74 signals were first extracted on  $N = 51$  brain areas from a home-made atlas based on published  
75 ones [24, 25] after normalization of anatomical images and co-registration of fMRIs (Appendix B)  
76 and signal extraction for each area with weighted averaging on voxels (Appendix D). SV and  
77 BOLD signals are decomposed with wavelet transforms (Appendix B) with exclusion of samples  
78 contaminated by movements (Appendix E). Correlations are performed on these signals to evaluate  
79 FC (Appendix G).

## 80 2.4. Statistical analysis

81 To test for the difference of values between groups with unknown distributions, two-sided  
82 Wilcoxon rank-sum tests, with an appropriate hypothesis for paired samples or independence  
83 of samples are applied. The  $p$ -value is computed, and the hypothesis  $H_0$  of the same mean of  
84 populations is accepted or rejected at different levels: no reject (-), reject at 5 % (+), reject at 1  
85 % ( $\times$ ), reject at 0.1 % ( $\dagger$ ). Pairwise comparisons are realized for different groups and a sequential  
86 notation is given for each group as compared to the other. A small circle ( $\circ$ ) is used to indicate the  
87 position of the current variable. Not available comparisons are indicated by small bullets ( $\bullet$ ). For  
88 example, a comparison of the mean of three populations G1, G2, G3, with: G1 and G2 significantly  
89 different at  $1e-3$ ; G2, G3 at  $5e-2$ ; and G1, G3 not significantly different, will be noted with the  
90 three sequences : ( $\circ \dagger -$ ) for G1, ( $\dagger \circ +$ ) for G2, and ( $- + \circ$ ) for G3. When linear regressions are  
91 estimated on bivariate samples, the null hypothesis that the slope of the regression is 0 is tested  
92 with a Wald test using  $t$ -values. Significant correlation coefficients are obtained by computing  
93  $p$ -values based on ad-hoc hypothesis tests for correlations and wavelets [26, 27] and adjusting for  
94 multiple corrections. (see also Appendix G).

## 95 2.5. Data availability

96 Data and codes are available at DOI 10.5281/zenodo.2452871.

## 97 3. Results

### 98 3.1. Effects of anesthesia on SV and baseline CBF

99 Number of animals per group, means (m.) and standard deviations (sd.) of SV measured  
100 during the fMRI protocol are given in Table 1 and distributions of values with box plots are given  
101 in Fig. 1.

102 SV have usual ranges observed during anesthesia on rats, with high HR for Iso-L, unstable HR  
103 for Eto-L and Ure-L (sd.  $> 15\%$  m.), high RR for Ure-L, and difficulty to measure peripheral  
104 oxygen saturation as a consequence of the cooling of peripheral limbs for Ure-L (missing values  
105 and one individual with low values, see Fig. 1d). Homogeneous values of variables are observed  
106 within groups (scatter plots of pairs of SV given in Fig. I.20, Fig. I.21 and Fig. I.22). There is a  
107 relationship between HR and Temp ( $R = 0.56$ ,  $p < 0.01$ ). Baseline CBF is significantly different  
108 between groups (Fig. 1g). The highest baseline CBF is observed under isoflurane (Iso-L: 67, Iso-W:  
109 101 ml / 100 g / min), with an influence of the rat strain. The lowest baseline CBF is obtained  
110 for Med-L (19 ml / 100 g / min), in line with previous reports [28, 29, 30]. Relationships between  
111 baseline CBF and SV are only significant for HR ( $R = 0.56$ , Fig. I.20) and Temp ( $R = 0.49$ ,  
112 Fig. I.22). Overall, anesthetics change SV and baseline CBF values with induced steady states for  
113 each group.

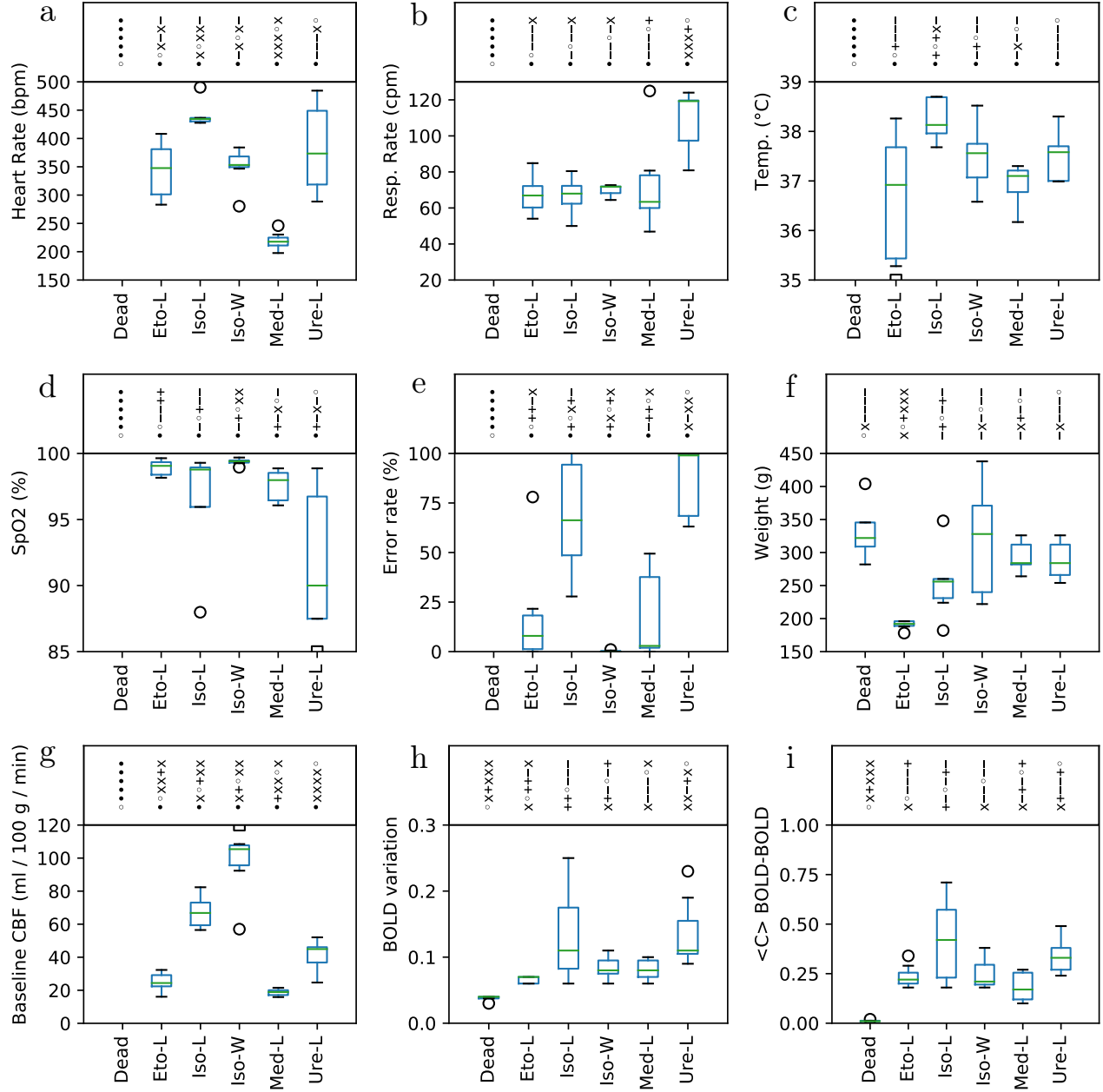


Figure 1: Distributions of variables per group with box plots. The distributions are represented by boxes and whiskers: green bars indicate median values, upper and lower sides of the boxes indicate the 1st and 3rd quartiles, whisker bars are given for values under 1.5 times the interquartile limits and circle markers indicate outlier values outside this interval. Square markers indicate outlier values out of the plot range. Above each whisker plot, the *p*-value of the test of comparison of distributions with the other groups (unpaired Wilcoxon tests). Markers indicate the level of significance of the *p*-values: - > 0.05 > + > 0.01 > x > 0.001 > †. The position of the reference group is indicated by o and unavailable comparison by •.

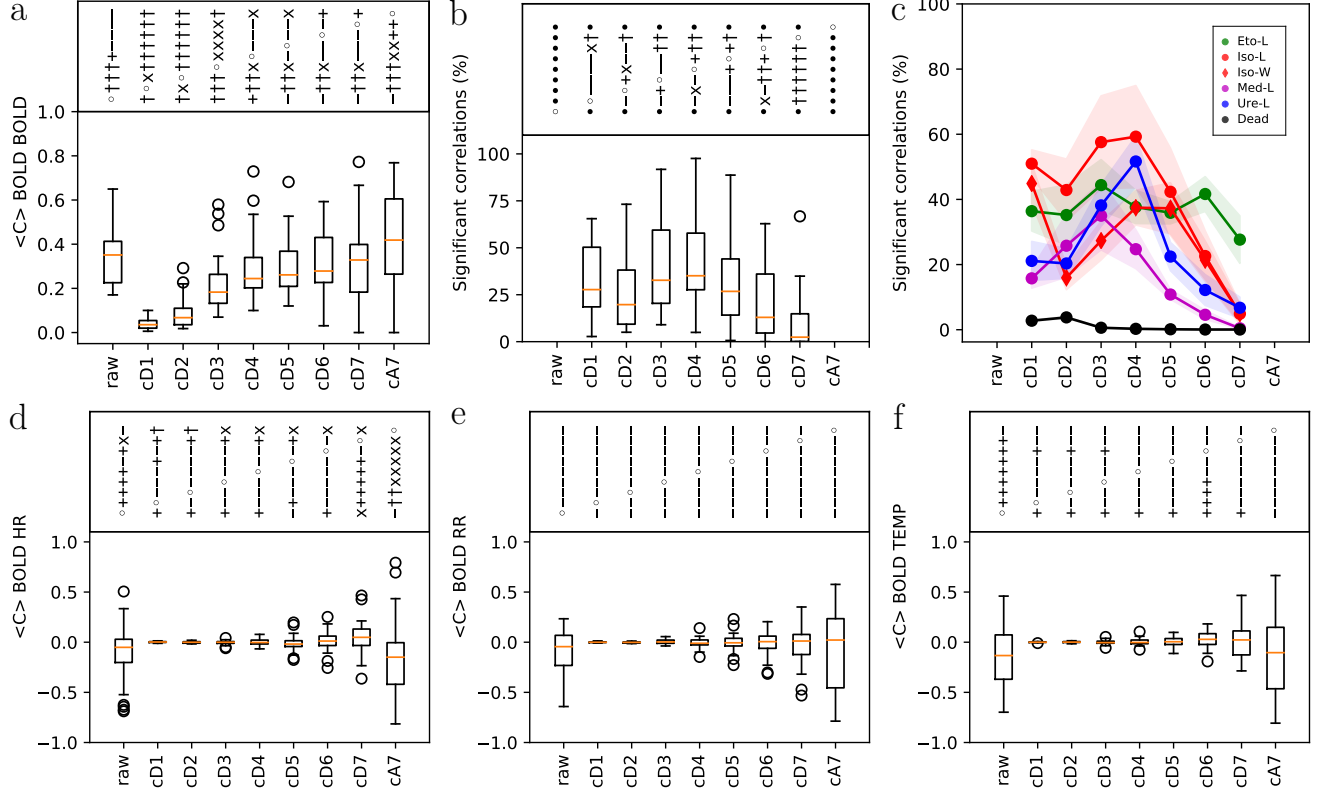


Figure 2: Distributions of correlations by frequency bands. a) Whisker plots of  $\langle C \rangle$  BOLD-BOLD, the mean functional connectivity between BOLD signal pairs at a given frequency band for all 51 brain areas, averaged across all animals, as a function of frequency band. b-c) Global and per group distributions of the percentage of significant correlations per frequency band. d-f) Whiskers plots for  $\langle C \rangle$  BOLD-HR  $\langle C \rangle$  BOLD-RR and  $\langle C \rangle$  BOLD-Temp, the average correlation between heart rate (HR), respiratory rate (RR) and temperature (Temp.) signal, and BOLD signals, at a given frequency band.

### 114 3.2. SV, BOLD signals and FC across frequency bands

115 fMRI BOLD signals were first extracted on  $N = 51$  brain areas from a home-made atlas  
 116 based on published ones [24, 25] (see materials and methods and Appendix C for details). As  
 117 BOLD signals and SV were simultaneously recorded, we evaluated the relationship between BOLD  
 118 signals with themselves (BOLD-BOLD) and with SV (BOLD-HR, BOLD-RR and BOLD-Temp)  
 119 for different frequency bands after motion filtering (see materials and methods Appendix E for  
 120 details). Frequency bands are obtained using dyadic wavelet transforms. The decomposition is  
 121 done on rs-fMRI raw signals (raw) to yield signals corresponding to detail coefficients at different  
 122 scales (cD1 - cD7) and one signal corresponding to approximation coefficients (cA7). The frequency  
 123 bands are, in Hz:  $cA7 < 0.008 < cD7 < 0.016 < cD6 < 0.031 < cD5 < 0.063 < cD4 < 0.125 <$   
 124  $cD3 < 0.25 < cD2 < 0.5 < cD1 < 1$ . Let  $C_{ij}$  be the BOLD-BOLD correlation coefficient between  
 125 signal from area  $i$  and signal from area  $j$ . The functional connectivity strength (FCS)<sup>2</sup> for node  $i$  is  
 126 defined as  $FCS_i = 1/(N - 1) \sum_{j,j \neq i} C_{ij}$ . The mean FCS is then defined as  $\langle C \rangle \text{ BOLD - BOLD} =$   
 127  $1/N \sum_i FCS_i$ . For SV signals, we defined  $\langle C \rangle \text{ BOLD - SV} = 1/N \sum_j C_{svj}$  where  $C_{svj}$  is the  
 128 BOLD-SV correlation between SV signal and signal from area  $j$ . Each of these quantities are  
 129 computed for each frequency band using the corresponding wavelet coefficients.

130 Fig. 2a represents the distributions of  $\langle C \rangle$  BOLD-BOLD in function of frequency bands over all  
 131 animals.  $\langle C \rangle$  BOLD-BOLD increases with the frequency bands with significant differences from  
 132 cD1 to cD3 with other scales, and median close to 0 for cD1 - cD2. Significant BOLD-BOLD  
 133 correlation coefficients are obtained by computing  $p$ -values based on ad-hoc hypothesis tests for  
 134 correlations and wavelets [26, 27] and adjusting for multiple corrections (see Appendix G for  
 135 details). Fig. 2b,c shows a maximum of significant correlations for the frequency band cD4, where  
 136 mean  $\pm$  standard error of the mean ( $M \pm \text{SEM}$ ) are represented for each anesthetic in Fig. 2c. To  
 137 validate our method, a group of dead rats (Dead) was created by sacrificing 4 animals. Almost no  
 138 significant BOLD-BOLD correlations are obtained on this group. Fig. 2d-f shows that the variance  
 139 of  $\langle C \rangle$  BOLD-HR,  $\langle C \rangle$  BOLD-RR and  $\langle C \rangle$  BOLD-Temp increases with the observed frequency  
 140 band. Interestingly, as frequency band increases, the median  $\langle C \rangle$  BOLD-BOLD increases faster  
 141 than the variance of  $\langle C \rangle$  BOLD-SV. This suggests that there is a frequency band for which the  
 142 ratio between the median  $\langle C \rangle$  BOLD-BOLD and the variance of  $\langle C \rangle$  BOLD-SV is optimal. This  
 143 band corresponds to a maximum in edges detection based on signal and not noise, as confirmed by  
 144 the number of significant BOLD-BOLD correlation coefficients per animal given in Fig. 2c. cD4  
 145 seems a good compromise for all groups, even though for some anesthetics the same number of  
 146 significant correlations can be found for cD3 to cD5 (0.031 - 0.25 Hz). The cD4 band (0.063 -  
 147 0.125 Hz) is thus retained to compute BOLD-BOLD correlations. In the remainder of this paper,  
 148 BOLD signal corresponds to the BOLD signal filtered in this band.

### 149 3.3. BOLD variations and FCS are independent of baseline CBF

150 To evaluate the impact of baseline CBF on BOLD signals, we computed the standard deviation  
 151 of the BOLD signal for each brain area, denoted as BOLD variation. Fig. 3a shows the BOLD  
 152 variation as a function of baseline CBF, binned per CBF intervals of 10 ml / 100 g / min and  
 153 averaged across rats per group, with values given in  $M \pm \text{SEM}$ . Despite the broad range of baseline  
 154 CBF values, almost flat curves are observed for each anesthetic over a large range of CBF values.  
 155 Similar results are obtained for different frequency bands (see Fig. I.31). Fig. 3b shows similar

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<sup>2</sup>This definition is related to the one proposed in [31] or the normalized version of vertex (node) strength or weighted degree proposed in [32, 33]

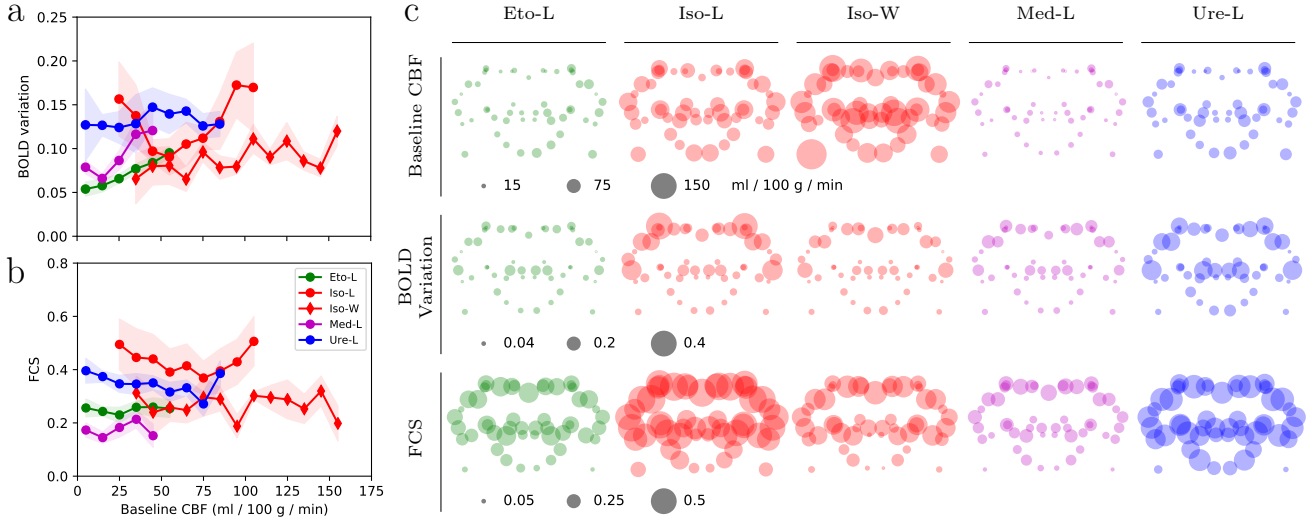


Figure 3: Relationships between baseline CBF, BOLD variation and FCS. a) Average values of BOLD variation per CBF bin and per group with at least 4 animals. b) Functional connectivity strength (FCS) per CBF bin and per group with at least 4 animals. c) Comparison per group on each node for baseline CBF, BOLD variation and FCS. Each area is represented by a node located at its center of gravity on a coronal projection. Node diameter is related to parameter value.

almost flat curves obtained for FCS as a function of baseline CBF. This indicates that average values of BOLD variation and FCS do not depend on the average baseline CBF values. There is no significant relationship between baseline CBF and BOLD variation or  $\langle C \rangle$  BOLD-BOLD (Fig. I.26), but there is a significant relationship between BOLD variation and  $\langle C \rangle$  BOLD-BOLD ( $R = 0.87$ ,  $p < 0.001$ ) (Fig. I.27). Fig. 3c shows, per group, a coronal view of baseline CBF, BOLD variation and FCS in the brain. It further suggests that CBF, BOLD variation and FCS are not tightly linked. For example, even if CBF is high for Iso-W, BOLD variation is low. For high baseline CBF values observed in the Iso groups, the measures in BOLD are high for Iso-L and medium for Iso-W. Eto-L and Med-L produces similar patterns, with low values for all three variables. Altogether, the FCS patterns seem similar across all anesthetics, not sensitive to either CBF or BOLD variation.

#### 3.4. Anesthetics target specific brain areas and reshape distribution of connections

For each group of rats, average correlation matrices are computed. Based on our results in Fig. 2c, edges not significantly different to zero correlations are removed. FCS per group and area are presented in Fig. 4a with FCS computed here using  $FCS_i = \frac{1}{N_i} \sum_{j \in S_i} C_{ij}$  with  $S_i$  the set of significant correlations for area  $i$  and  $N_i$  the number of elements in  $S_i$ . This approach is validated on the group of dead rats where no connections remain. When computing the average correlation matrices for the other groups of rats, 9 areas only are not significantly different from noise. This means that at most 9 (/51) brain areas, or 6 (/26) by grouping bilateral areas can not be differentiated from random signals, whatever the anesthetic. Hence, we can not conclude on their specific connections with other areas. On average, sensory (S1, S1BF, S2), motor (M1, M2) and cingulate cortex (ACC) areas have the highest correlation values. This is emphasized by Fig. 4b where areas are ranked from the most connected area to the least one. Depending on the anesthetic, some areas are more connected in some groups (e.g. V1 in Iso-L, Ins in Iso-W, Th in

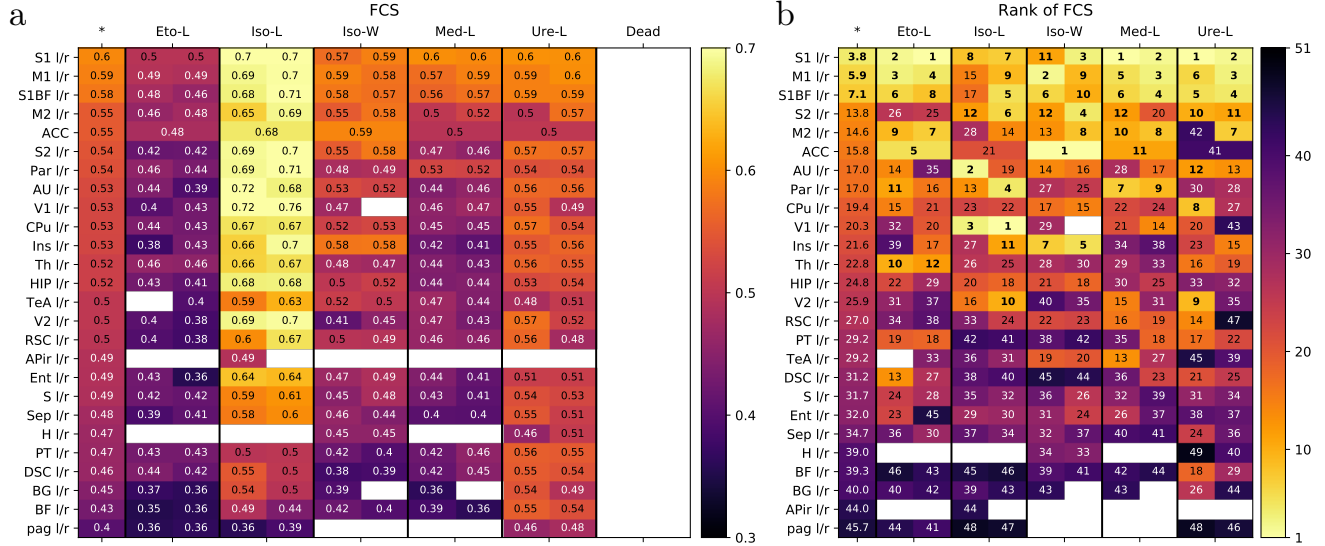


Figure 4: Graph measures and representations per group of average matrices. Average was performed on significant correlations at a symmetrical level  $\alpha = 5\%$  (Bonferroni corrections  $p=2e-5$ ) and if present in a minimum of 4 animals per group. (a) FCS. Each area is represented by two columns (left and right hemispheres), except for ACC which is central and for column \* which is the average of anesthetized groups. Areas are sorted from this column. Unavailable FCS from areas with no detected edges are not plotted (white). (b) FCS rank per group, sorted from highest to lowest. A rank of 1 indicates that the area has the highest FCS in its group. Areas showing the lowest ranks (first quartile) are in bold. The first column \* is the average of anesthetized groups. Areas are sorted from this column. Notations: l: left, r: right, ACC: anterior cingulate cortex, APir: amygdalopiriform transition area, AU: auditory cortex, BF: basal forebrain region, BG: basal ganglia, CPu: caudate-putamen striatum, DSC: superior colliculus, Ent: entorhinal area, Ins: insular cortex, H: hypothalamic region, HIP: hippocampus, M1: primary motor cortex, M2: supplementary motor cortex, pag: periaqueductal gray, Par: parietal association cortex, PT: prefrontal region, RSC: retrosplenial cortex, S: subiculum, S1: somatosensory 1, S1BF: somatosensory 1 barrel field, S2: somatosensory 2, Sep: septal region, TeA: temporal cortex association area, Th: thalamus, V1: primary visual cortex, V2: secondary visual cortex.

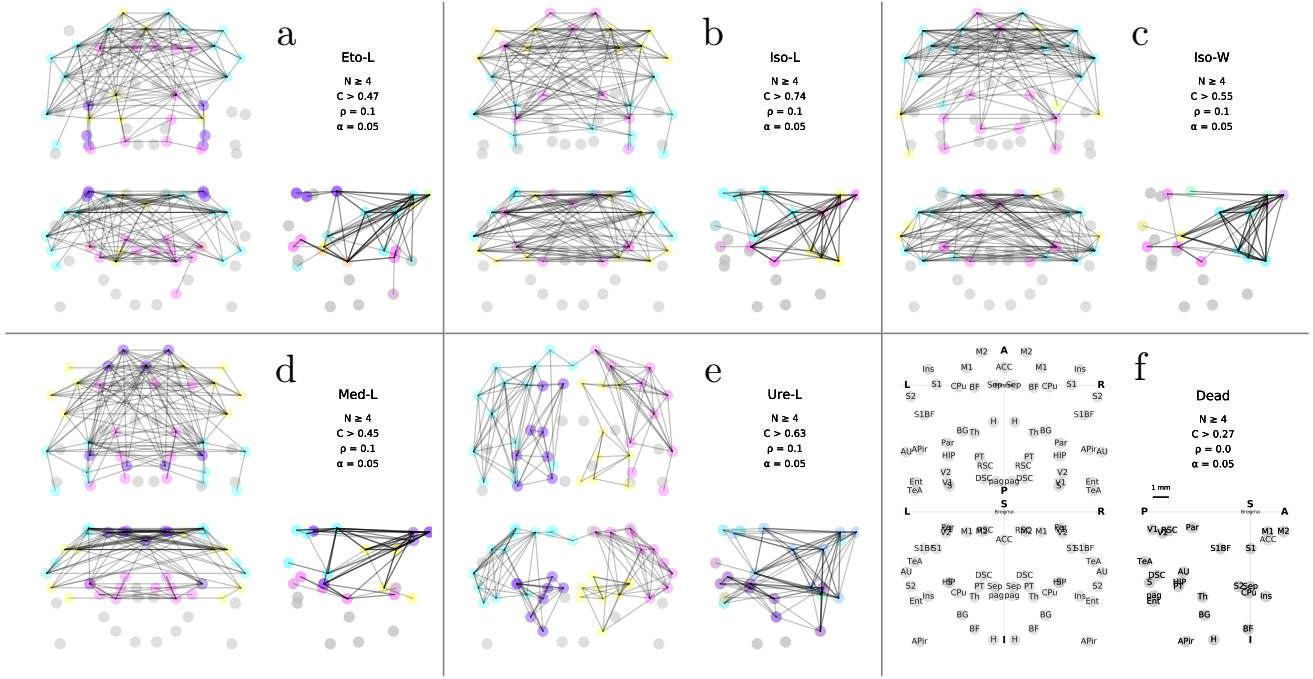


Figure 5: Graph representations of average matrices per group. Average was performed as in Fig. 4. (a)-(f) Graphs based on the average correlation matrices obtained with the frequency band cD4 for all group. The 127 most correlated values (density  $\rho = 0.1$ ) are plotted. Gray nodes represent unconnected nodes and nodes with the same arbitrary color belong to the same community. For each group, the number of animals, the lowest correlation value used in the graph, and the correlation significance threshold are mentioned. (f) The Dead group shows no significant connections. Notations same as Fig. 4 with L-R: left-right axis, A-P: antero-posterior axis, I-S: inferior-superior axis.

Eto-L) or less connected (e.g. Acc in Ure-L, S2 and AU r in Eto-L). Asymmetries between left and right areas may also be observed (e.g. Ins and AU in Eto-L, M2 in Iso-L and Ure-L). These observations should be mitigated by the fact that FCS values show a moderate but significant relationship with the area location in the MRI device ( $R=-0.68$ ,  $p < 0.001$ ), and, at a lesser level, with the area size ( $R=0.56$ ,  $p < 0.01$ ) (Appendix H), with low values of FCS obtained for small and far from the coil areas, such as TeA. After verifying that correlations did not originate from spurious neighboring signal contamination by evaluating the presence of significant long-range correlations in each group (Fig. H.16), graphs were constructed per group to highlight local differences in connectivity.

Fig. 5 displays the thresholded correlations with a density<sup>3</sup>  $\rho = 0.1$ . Nodes communities detected by clustering are given in color (details in Appendix B). The graphs of anesthetized groups are structured with the anterior cingulate cortex (ACC) taking a central place in connectivity. Among the acquired nodes, nodes with low FCS seem less connected for some groups (H, BF, BG, APir, pag). There is also an obvious loss of the inter-hemispheric connections in the Ure-L group and a loss of the cortical-subcortical connections in the Med-L group. This is further confirmed by individual graphs and other representations proposed in Fig. I.32 to Fig. I.37 and Fig. I.38.

Finally, we summarized the impact of anesthetics from graphs of Fig. 5 by representing the ratio of connections between ACC, cortical (c), subcortical (s), interhemispheric (inter) or intrahemi-

<sup>3</sup>The number of retained edges over the number of all possible edges



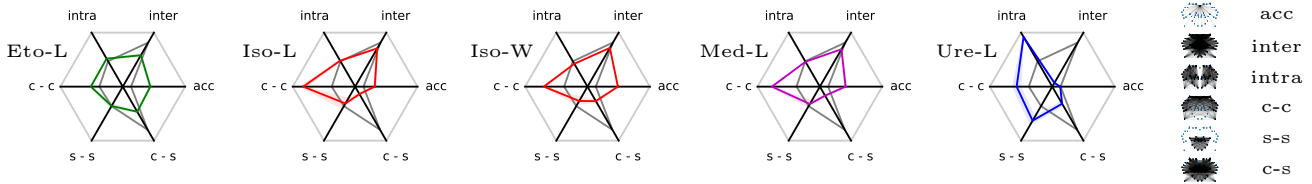


Figure 6: Radar representations of average matrices per group. Average was performed as in Fig. 4. Radar plots represent the distributions of connections for the graphs presented in Fig. 5, obtained at a density  $\rho = 0.1$ . Gray curve represents percentages for the fully connected graph. Notations: acc: anterior cingulate cortex, inter: interhemispheric, intra: intrahemispheric, c: cortical, s: subcortical.

spheric (intra) areas. Fig. 6 shows the spatial reorganization of the 10 % strongest correlations, given by graphs at density  $\rho = 0.1$ . We observe low ACC FC for Ure-L, very low inter FC for Ure-L, low inter FC for Eto-L, and low c-s FC for Iso-L, Med-L and Iso-W (see Appendix H for values and comparison with other densities).

In summary, depending on the anesthetic and the animal strain, 25 to 60 % of the total possible connections are significant with at least 80 % of areas connected to at least one other area, with reordering of the strongest connections.

## 4. Discussion

### 4.1. Summary of findings

After observing that SV and CBF under anesthesia were stable, we introduced an approach based on wavelet decomposition to identify the optimal frequency bands to analyze FC and the number of significant correlations between areas. This approach limits the interaction with SV and improves the signal to noise ratio for the detection of significant graph edges. Using this method, and data including baseline CBF and rs-fMRI acquired in rats under several anesthetic conditions, we observed that, in the low frequency band 0.06-0.12 Hz, BOLD variation and FCS are independent of baseline CBF. Overall, the vast majority of brain areas are connected together with anesthetic dependent connectivity patterns.

### 4.2. Impact of anesthetics on SV, choice of BOLD frequency bands and FC

SV values reported in this study are inline with previous reports using comparable anesthetics and preparations [34, 35, 3, 36, 4]. Fig. 2d-f indicate that, whatever the baseline SV, fluctuations of SV in some frequency bands (cD3 - cD5: 0.031 - 0.25 Hz) do not correlate with spontaneous BOLD signals during the 30 min acquisition. We also evaluated whether the SV averaged across the acquisition duration and the BOLD variation averaged in the same way were correlated when considering all animals. With this approach, correlations were observed between average SV and average BOLD variations (Fig. 3; e.g. Temp. vs BOLD variation), possibly resulting from the increased variability of SV fluctuations at frequencies below 0.031 Hz. Altogether, despite the broad range of SV conditions produced by the use of different anesthetics, SV fluctuations did not contaminate BOLD signal in the 0.031 - 0.25 Hz band for a 30 min acquisition.

Modifications on signals may also be related to movements of the animals. Since animals were restrained with teeth and ear bars, the contamination of the BOLD signal is less probable than in human studies. Nevertheless, motions can occur and a classical scrubbing method with boxcar filtering [37] has been applied, taking into account HRF and wavelet support at different scales (see Appendix E). Alternative methods to filter out SV and movements exist and are recommended by

other studies [38], but they lead to complex modifications of signal that may prevent a quantitative analysis of correlation coefficients and lead to controversial results, as for example with global signal regression (GSR) [39]. Transformations of correlations are also complex to interpret when working with non-Gaussian processes and lead to distributions of correlations that are difficult to compare [5, 40, 41, 42, 6]. Without any transformations, the distributions of correlations (Fig. I.29) are comparable with the ones presented in [43, 44, 45] and show influences of anesthetics on  $\langle C \rangle$ . It would however be of interest to further investigate and compare these different transformations.

The study of the number of significant correlations as a function of the frequency band seems a useful step to optimize and validate the FC processing pipeline. We advocate the use of both dead rats [4] and random simulations [27, 46]. Indeed, empty graphs may be obtained when the signal to noise ratio is too low (Fig. 5), and thereby enhance contrast for specific communities and networks. The selected frequency band (cD4: 0.062 - 0.125 Hz), determined by experimental constraints and mathematical framework, was narrower than previous experimental reports but included, like most reports, frequencies below 0.1 Hz (see Appendix F). Bandwidths could be further adjusted for example by merging some of them, also based on observations of the power spectrum of signals (Appendix F) as given in [4, 44, 16]. Since the velocity of the hemodynamic response depends on baseline CBF [18], the optimal frequency band could depend on the type of anesthetic or on the animal strain [15, 4, 16]. Since no correlations between baseline CBF and BOLD variations in the cD4 band were observed, our data do not demonstrate this point, in line with a previous report showing that spontaneous sinusoidal oscillations may exist over a range of anesthesia depths [47]. This is also inline with BOLD variations caused by arteriole vasomotor variations [48] under the control of locally regulated vasoactive substances [49, 50]. The method we propose could be adjusted to explore narrower frequency bands and thereby smaller change in hemodynamic response function, based on longer rs-fMRI acquisitions.

#### 4.3. Impact of anesthetics on baseline CBF, BOLD variations, and $\langle C \rangle$ BOLD-BOLD

The choice of anesthetic and of animal strain changed the baseline CBF, a parameter correlated to mean HR and mean Temp (Fig. I.30). Baseline CBF was however independent of BOLD variations and of FCS (Fig. 3). This is in line with [51] but seems in contradiction with previous reports, which demonstrated that an increase in baseline CBF decreases the BOLD signal amplitude, comparable to the BOLD variation in our study [18]. The difference with [18] is that a change is observed in one given brain area, whereas all brain areas with the same CBF are averaged in our study. Mean  $\langle C \rangle$  BOLD-BOLD were however dependent on HR and Temp, suggesting that this parameter is not fully independent of baseline physiology. Mean arterial pressure could also modulate the BOLD variation [52] but was not evaluated in this study. Altogether, extreme care should be taken to maintain normal and stable physiological conditions while performing rs-fMRI.

#### 4.4. Impact of anesthetics on brain connectivity

Our proposed pipeline using anesthetics extracts connectivity matrices of brain rats with 25-60 % significant connections (Fig. 2c), containing 42/51 (80 %) areas significantly connected with at least one other area (Fig. 4). This range of 25 % to 60 % of significant connections is consistent with density observations in the macaque monkey (up to 66 %, 29 areas), using a retrograde tracer analysis [53], and in mice (up to 36 %, 213 areas), using an anterograde tracer [45]. By acquiring good quality data and using robust methods, it seems therefore possible to observe fMRI graphs in which most brain areas remain functionally active, even when the rats are anesthetized. The few apparent not significantly connected areas (H, BF, BG, APir, pag) are located far from the receive surface coil (decrease of FCS in function of distances from Bregma given in Appendix H)

and close to large vessels or cerebro-spinal fluid, two sources of noise. The lower signal to noise ratios for these areas could contribute to the absence of significant FCS in some areas such as TeA or APir. The position of the left and right areas near the ears with potential distortions (see Fig.B.8) can also add noise in these areas, but no correlations were observed between FCS and medio-lateral locations of areas (Appendix H). The graph approach provides a global view of the FC and characteristic patterns, with differences in the global organization of the brain connectivity. This is in line with previous studies [4, 54, 3, 21, 6, 43] where the authors used seed based analysis or independent component analysis (ICA). In this work, the impact of anesthetics is visible at the regional level with communities (Fig. 5) partially related to networks such as the default mode network (DMN) or the resting state network (RSN) [55, 43, 38]. Compared to ICA approaches, which highlight the most orthogonal networks, the graph approach used in this study rather seeks for all potential connections [56, 57]. Seed Based Analysis (SBA) also promotes a limited number of connections due to the limited number of seeds. Graphs may be seen as an SBA approach in which every region is a seed, with FCS giving the strength of the seed (Fig. 4). In contrast to graph analysis, ICA or SBA approaches usually distinguish segregated networks. Our results may not appear in agreement with these approaches, and a study using the same data but with different analysis methods is required to conclude whether they lead to similar or complementary conclusions concerning biological processes. The impact of Urethane and Isoflurane on M1, S1 and CPu [21] is shown at the regional level, but also at an intermediate level where balance of connections between cortico-cortical areas, cortico-subcortical areas, or intrahemispheric and interhemispheric areas are modified depending on anesthetics. The study of the effects of anaesthesia on bilateral connectivities in rodents is an active field of research [58], with Medetomidine, which may reduce thalamocortical transmission [35] (Fig. 6, Tab. H.3 also observed for Isoflurane in this study), but also bilateral connectivity [3, 58] (not observed here), and Urethane, which may decrease the strength of bilateral connections [4, 21] (Fig. 6, Tab. H.3 in this study) or even induce fluctuating connectivity states with periods on the order of a few minutes to tens of minutes as reported in [59]. The influence of anaesthetic dose, rodent strain, animal state and details of the analysis method on bilateral connectivity under anaesthesia requires further investigation. Concerning these observations, our results are consistent with literature. However, the different communities extracted with our method are not easily comparable with those obtained with commonly used GSR or ICA. One could expect that our method would show communities comparable to previously reported functional networks such as DMN (including ACC, RSC, and Tea<sup>4</sup>) with stability observed across different anesthetics regimes [38]. Here, we observe that there might be connections between ICA-reported networks. For example, ACC is part of the DMN. It is also known to be involved in several processes such as the balanced between behaviors, autonomous functions or nociception, and known to have a lot of connections with other areas including sensory and motor areas [60, 61, 62]. ACC, as defined in our study, can therefore be involved in different networks. Some comparisons between graph-based analyses and the effect of GSR and ICA are given in [44], but it would be of interest to perform an entire study on the same dataset to compare and enhance the complementarities of the different methods.

#### 4.5. Main limitations

To overcome the limited brain coverage (10 mm that excluded the cerebellum and the olfactory bulb), simultaneous multislice MRI should be developed at the preclinical level. The number of

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<sup>4</sup>with prefrontal cortex area (PFC) not included in the study because this area was not available in several recordings

animals per group is limited ( $n = 6$  or  $7$ ) and some groups exhibit heterogeneity. Main findings are however related to all live animals ( $n = 34$ ) and the impact of physiological variations was carefully analyzed. The anesthetic dose in this study were standard ones but not optimal ones related to anesthesia depth, leading to comparisons between drugs that should be mitigated. It is indeed expected that the anesthetic dose also leads to different functional connectivity patterns, as observed with isoflurane [14, 3, 44, 21], and should be evaluated in other studies in association to the search of an optimal dose. Besides, further studies are required to account for the contribution of the metabolic effects such as oxygen and glucose consumption and electrical activity [63, 64, 36]. Lastly, nothing can be interpreted from the remaining 20 % of the areas that are not significantly connected.

## 5. Conclusions

In summary, this study introduces a methodological approach to evaluate the influence of different anesthetics on rats during anesthesia. Using a rigorous mathematical framework based on wavelets-based spectral filtering, we have shown that systemic variables and the baseline CBF, in the range evaluated in this study, have no influence on the slow fluctuations of BOLD signals and on FC. Moreover, most brain areas remained functionally active under the anesthetics used in this study with density of the graphs in the range between 25 % to 60 %. This demonstrated, we have proposed measures that highlight the influence of the different anesthetics on FC, at the level of brain areas or between them. Future work could use the proposed methodological framework with increased populations, adjusted anesthetics concentrations or refined spectral bands to better tune the effects of anesthetics on the whole brain, on functional connectivities or on specific target areas.

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## Competing Interests

The authors have no conflict of interests.

## Author contributions

Design research: ELB, SA, GJPCB / Performed research: TH, NC, MF, VC / Contributed analytic tools : GJPCB, SA, MF, VC, CDM / Analyzed data : GJPCB, ELB, SA / Wrote the paper: GJPCB, ELB, SA.

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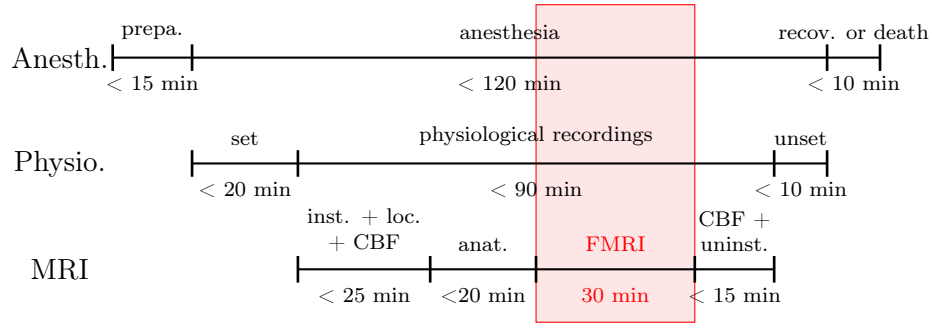


Figure B.7: Experimental protocol. Notations: Anesthesia (Anesth.), SV (Physio.), Magnetic resonance imaging (MRI), preparation of the animal (prepa.), installation of the animal in the scanner (inst.), position verification with a localizer sequence (loc.), cerebral perfusion sequence (CBF), anatomical sequence (anat.), rs-fMRI sequence (FMRI), uninstallation of the animal (uninst.), recovery or death of the animal (recov. or death)

## Appendix A. Anesthetics used in rodents

A list of the anesthetics evaluated on rodents is given here with associated references:

- $\alpha$ -chloralose [65, 15, 66, 21, 67, 6, 68, 14],
- diazepam (benzodiazepine) [69],
- etomidate [19],
- fentanyl [8],
- halogeneous derivates [70, 71, 15, 4, 8, 63, 21, 72, 73, 74, 42, 44] [75, 76, 77, 69, 6, 78, 43, 79, 64, 80, 81, 16, 14],
- ketamine [5, 70, 41],
- medetomidine [5, 70, 40, 41, 4, 3, 13, 72, 55, 76, 82, 58, 6, 78, 83, 84, 85, 16, 14, 86],
- MPEP (mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine [64],
- pentobarbital [8, 87],
- propofol [4, 88, 6],
- urethane (carbamate) [4, 21, 69, 6].

Animals can also received curare (pancuronium bromide) infusion [44, 88, 83, 68, 86] to relax muscle and avoid movements or paralyzing agent (gallamine triethiodide) [43].

Elements on what is known about the effects on brain functions, neurons interactions, ionic channels affinities and physiological interactions can be found for example in [49].

## Appendix B. Supplementary Materials

### Appendix B.1. Experimental protocol

A scheme of the experimental setup used to record data from anesthetized rats is proposed Fig. B.7 and can be summarized as follows. For the anesthesia, a preparation of the animal is realized in an induction room. Anesthesia generally lasted less than 2 hours. At the end of the protocol the animal is kept alive for another study or euthanized, if in the Ure group or randomly assigned to the Dead group. For the systemic variables, also denoted SV, sensors are set and unset before and after the recordings. For magnetic resonance imaging, the animal is installed in the scanner and positions are verified with localizer sequences. A perfusion sequence and an anatomical sequence are acquired before the fMRI sequence which lasts 30 min. Then, another perfusion sequence (CBF) is acquired, followed by the uninstallation of the animal.

## 733 *Appendix B.2. Anesthesia*

734 At the beginning of the experiment, animals receive a gaseous mixture, composed of isoflurane  
735 and a mixture of 80 % air and 20 % oxygen, inhaled through a funnel. A level of 5 % isoflurane is  
736 given during 2 min followed by a level of 2 % during 10 minutes. During the animal preparation,  
737 ears are filled with a mix of gel (ocry-gel) and wax, to limit air-related susceptibility artifacts in  
738 the images. After this common induction, four anesthetics are evaluated:

739 - Isoflurane (Iso): the level of isoflurane is set to 1 % to be in a regime showing persistent  
740 behavior of spontaneous BOLD fluctuations [89]. To evaluate the impact of strain, this anesthetic  
741 was evaluated in both Long-Evans and Wistar rats.

742 - Etomidate (Eto) (B. Braun, Melsungen, Germany) : An administration in two stages is  
743 performed with a bolus of 10 mg / kg / min (3 min) injected in association with isoflurane at 4%  
744 during initiation, followed by a continuous intravenous infusion at 0.5 mg / kg / min.

745 - Medetomidine (Med) (Domitor, medetomidine hydrochloride; Orion Corporation, Espoo, Fin-  
746 land): An administration in two stages is performed with a bolus of 0.24 mg/kg injected subcu-  
747 taneously in association with isoflurane at 4% during initiation, followed by a continuous infusion  
748 at 0.05 mg / kg / h.

749 - Urethane (Ure): A bolus of 1.25 g / kg is injected intraperitoneally. [90] The use of this  
750 anesthesia is well regulated and rats are euthanized at the end of the experiment because of the  
751 carcinogenic toxicity of this agent.

752 - A group of dead rats (Dead) was created by sacrificing 4 animals just before the experiment.

753 Overall, 6 groups were evaluated: Eto-L (n=7), Iso-L (n=6), Iso-W (n=7), Med-L (n=7), Ure-L  
754 (n=7), Dead (n=4).

## 755 *Appendix B.3. Systemic variables*

756 Two devices are used to monitor and record systemic variables (SV):

757 - A MouseOx Pulse oximeter (STARR Life Sciences Corp., Oakmont, PA, USA) with a rat  
758 sensor clipped on the right posterior paw of the animal. The system provides different measures:  
759 the peripheral arterial oxygen saturation (SpO<sub>2</sub>), the heart rate (heart rate). An error measure is  
760 also recorded to validate the measures recorded by the device. Only SV samples recorded by the  
761 oximeter and marked without errors are retained for the study;

762 - A SAII Trend (Small Animal Instruments Inc., Stony Brook, NY, USA) system is also used  
763 to record the temperature (Temp) from a rectal probe and the respiratory rate (resp. rate) from  
764 a small pneumatic pillow sensor. The error rate is computed as the ratio of the number of values  
765 recorded with code different from 0 (no error) on the oximeter to 3600, the number of samples  
766 during the whole rs-fMRI session. Clocks of the recording systems are verified at the beginning  
767 and at the end of the recordings, to follow a universal clock, noted, and eventually corrected.  
768 Measurements from the two systems are sampled at 1 Hz. No offline adjustments were necessary  
769 with the 1s precision. During the experiments a warm circulating water positioned below the  
770 animal is regulated to maintain the body temperature to a target level of 37°C. Dead animals were  
771 not monitored, not regulated, and were imaged one hour after sacrifice.

## 772 *Appendix B.4. MRI acquisitions*

773 Rats brains are scanned at 9.4 T with a volume transmit, 4-channel surface receive cross coil  
774 configuration (Paravision 6.0.1, Bruker, Ettlingen, Germany). After an anatomical T2w scan,  
775 cerebral blood flow (CBF) is mapped using pseudo continuous arterial spin labeling (pCASL;  
776 labeling duration 3000 ms, post-labeling delay 300 ms; TR=3500 ms; TE=9.7 ms; 20 averages;  
777 spin-echo 2D echo-planar readout with a spatial resolution 0.47 x 0.47 x 1.00 mm, gap 0.1 mm,

9 slices) following inter-pulse phase optimization as proposed in [23]. Then, resting state fMRI (rs-fMRI) was performed with a single shot echo-planar imaging with parameters set to: TR/TE = 500/20 ms, spatial resolution 0.47 x 0.47 x 1.00 mm, gap 0.1 mm, 9 slices, 3600 repetitions, duration 30 min. The volume of interest stands between 0 and -10 mm with respect to bregma and results in a volume of 64 x 64 x 9 voxels. Eventually, a second CBF map is acquired and averaged with the first CBF acquisition to obtain a mean CBF during the rs-fMRI experiment. Rats were strapped to the MRI insertion table with the head maintained with ear bars and a bite bar to prevent movements.

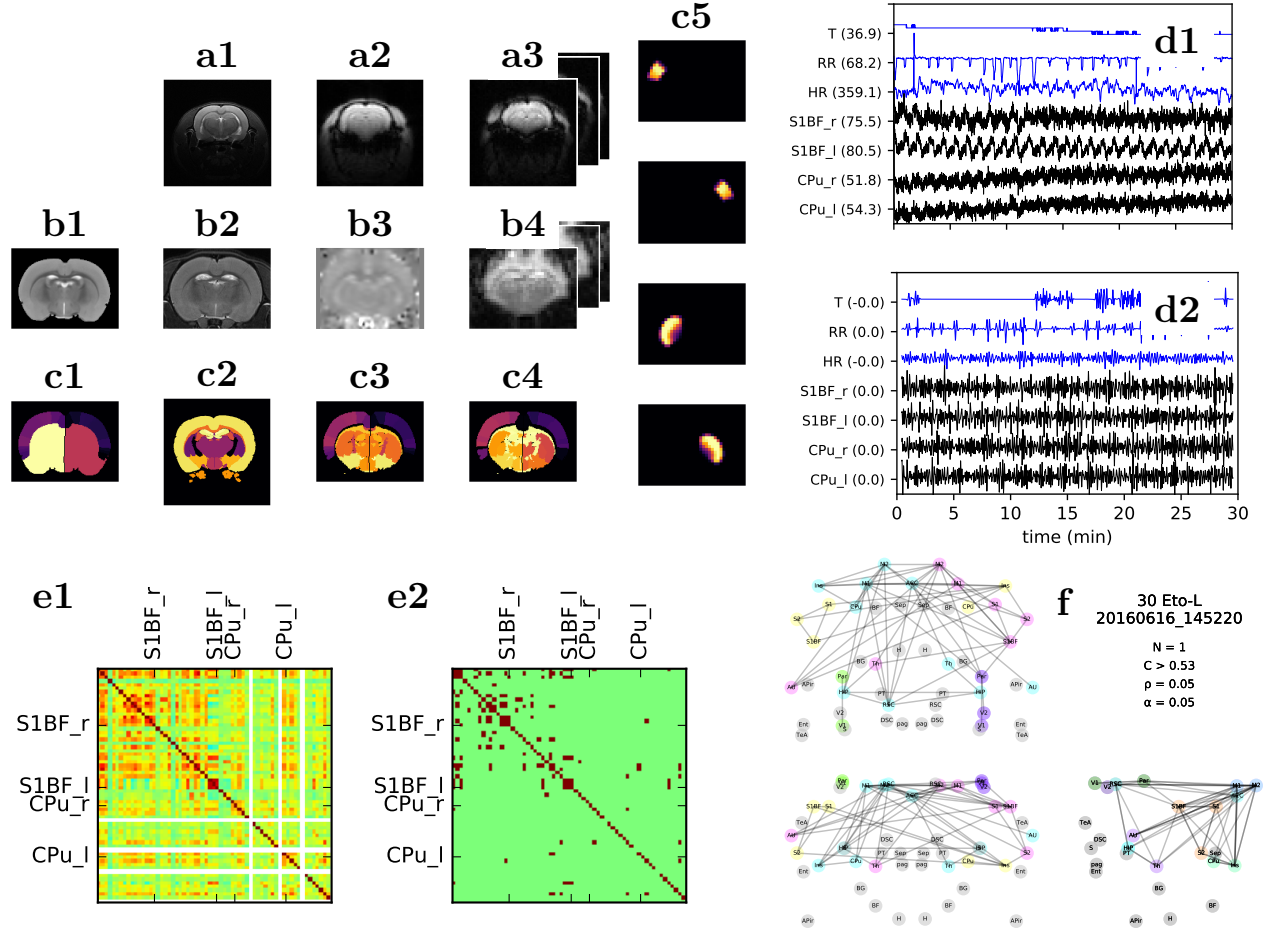
#### Appendix B.5. data processing

An overview of the data processing is given in Fig. B.8 for one individual, from the MR images to the graph representations of the brain activity and can be summarized as follows: fMRI BOLD signals are first extracted on  $N = 51$  brain areas from a home-made atlas (Appendix C) based on published ones [24, 25] after normalization of anatomical images and co-registration of fMRIs; Physiological and BOLD signals are motion corrected (Appendix E) and decomposed into different frequency bands with wavelet transforms described in the main text (see also Appendix F); Correlations are performed on these signals to evaluate FC.

##### Appendix B.5.1. Atlas creation, co-registration and normalization of MRI

The WHS atlas [25], focusing on subcortical areas is merged into the Tohoku atlas [24], focusing on cortical areas, to obtain our own atlas containing both cortical and subcortical areas (see Appendix C). All MRI recordings are aligned to the Tohoku template. Dimensions of voxels in the different spaces are: in the Tohoku space, 0.125 mm  $\times$  0.125 mm  $\times$  0.125 mm (0.002 mm<sup>3</sup>), 136  $\times$  102  $\times$  180 voxels; in the WHS space, 0.0391 mm  $\times$  0.0391 mm  $\times$  0.0391 mm, 512 by 1024  $\times$  512 voxels; for anatomical MRI, 0.117 mm  $\times$  0.117 mm  $\times$  0.8 mm, 256  $\times$  256  $\times$  27; for fMRI, 0.469 mm  $\times$  0.469 mm  $\times$  1.1 mm. Dimensions of anatomical images and rs-fMRI are multiplied by 10 to fit the dimension of the Tohoku atlas made to be compatible with algorithms of the Statistical Parametric Mapping software (SPM12) [91] defined for human images. The origin is manually set to match the origin of the Tohoku template i.e. to the bregma zero coordinate, at the apex of the most forward crossing fibers of the anterior commissure. A segmentation of the tissue of the anatomical image is computed and used to normalize the anatomical image to the Tohoku space. rs-fMRIs are realigned and resliced to the first image of the fMRI sequence. The coregistration variables are used to filter movements by analyzing framewise displacements [92] (Appendix E). A coregistration of the fMRIs on the anatomical image is computed and applied to all fMRIs while keeping the fMRI voxel size. For all these operations, a 4th degree B-spline interpolation is set. More precisely, these operations are realized using SPM 12 [91] with adaptation of rodents volumes to parameters of algorithms based on human volumes, following: 1 - change of origin to the Bregma site and modification of a size of a factor of 10 for anatomical and fMRI volumes. (batch 'Reorient Images' with scaling parameters set to 10), 2 - Normalization of anatomical images to template. (batch 'Old Segment' with Grey matter, White matter and cerebro-Spinal Fluid in the native space and tissue probability maps taken from Tohoku space. Gaussian per class [2 2 2 4] / Affine regularisation: average sized template, Warping regularisation: 1, warp frequency cutoff: 25, bias regularisation: light regularisation (0.001), bias FWHM 60 mm cutoff; sampling distance: 3) 3 - Correction of acquisition offset and coregister fMRI volumes to the anatomical volume (batch Realign: Estimate & Reslice / quality:0.9, separation: 4, smoothing (FWHM): 5, Num passes: register to first, interpolation: 2nd Degree B-Spline, Wrapping: no wrap, weighting: 0 files, resliced images: mean image only, interpolation: 4th degree B-spline, wrapping: no wrap, masking: mask images) (batch + Coregister: Estimate: Objective function: Normalised mutual information, separation: [4 2], Tolerances: [0.02, 0.02, 0.02, 0.001, 0.001,





Notations: LR: left right axis; IS: inferior superior axis; PA: posterior anterior axis., T: Temp, RR: resp.rate, HR: heart rate.

Figure B.8: Overview of the data processing to obtain FC graphs between brain areas on one rat: a-b) image transformations with reference on the Tohoku anatomic template (b1): raw (a1) and normalized (b2) anatomical MRI, raw (a2) and norm. (b3) perfusion MRI, raw (a3) and norm. (b4) fMRI; c) Atlas construction with reference on the Tohoku anatomic template (b1): Tohoku atlas (c1), WHS atlas (c2), merged atlas (c3), with left and right labeling (c4), low resolution area weights (c5) from top to bottom S1BF l, S1BF r, CPu l, CPu r; d) SV and fMRI signals extraction and wavelet decomposition: raw signals (d1) extracted from (b3) and (c5) and wavelet decomposition cD4 (d2); e) correlation matrix (e1) and thresholded one (e2) at density  $\rho = 5\%$ ; f) FC graph corresponding to (e2). In this graph, locations of nodes correspond to the centers of gravity of brain areas. Edges indicate the connectivity between two areas. The graph is projected on the three standard projections LR-IS, PA-IS, and PA-LR (LR: left right axis; IS: inferior superior axis; PA: posterior anterior axis).

0.001, 0.01, 0.01, 0.01, 0.001, 0.001, 0.001], Histogram smoothing fwhm: [7, 7], interp: 0, wrap: [0, 0, 0], mask: 0), 4 - Normalize fMRI images to template using transformation obtained in 2. (batch Old Normalise: Write / Bounding box: [[nan, nan, nan], [nan, nan, nan]], Voxel sizes [1.25, 1.25, 1.25], Interpolation: 0, Wrapping: [0, 0, 0].) At the end of the process, all CBF and all rs-fMRI images are in a volume of dimension  $36 \times 27 \times 21$ , with voxel size  $0.469 \times 0.469 \times 1.1$  mm ( $0.242 \text{ mm}^3$ ), the same resolution of the original fMRI but aligned and normalized to the Tohoku space.

### Appendix B.5.2. Cerebral blood flow maps

CBF maps are obtained using homemade Matlab routines based on [93, 23]. CBF values and rs-fMRI time courses are computed for each of the 51 areas of the atlas. If available, mean CBF values are extracted by averaging the values obtained before and after the rs-fMRI acquisition:  $(\text{CBF}_{\text{before}} + \text{CBF}_{\text{after}}) / 2$ .

### Appendix B.5.3. Signal extraction per area and wavelet decomposition

The processing of fMRI images to extract signals from atlas areas is done with Python using the Nipy package [94] and home-made algorithms. The signal of an area is computed as the weighted summation of signals across all the voxels from that area (see Appendix D for details). rs-fMRI time course of an area (raw signal), is decomposed with a maximal overlap discrete wavelet transform (modwt) at different scales and Daubechies orthonormal compactly supported wavelets of length  $L=8$ , using the wavedec package [27]. Details coefficients cD1 to cD7, and an approximation coefficient cA7 that captures the polynomial trends contained in the signal are extracted. This last is generally a source of noise in observations. The limits of the frequency band  $j$  is obtained by dividing the sampling frequency (2 Hz), by  $2^{j+1}$  and  $2^j$ . In our study,  $j$  varies from 1 to 7 leading to discard details  $< 0.008$  Hz where spectrums become flat (see Appendix F).

### Appendix B.5.4. Correlation of signals, graph creation and clustering

The functional connectivity is obtained by computing the pairwise correlation  $C_{ij}$  between area  $i$  and  $j$  of the BOLD signals filtered at different frequency bands [27] denoted  $x_i$  and  $x_j$ . It is given by:

$$C(i, j) = \frac{\frac{1}{N} \sum_{k=1}^N x_{ci}(k) x_{cj}(k)}{(\frac{1}{N} \sum_{k=1}^N x_{ci}^2(k) \frac{1}{N} \sum_{k=1}^N x_{cj}^2(k))^{1/2}} \quad (\text{B.1})$$

with  $x_{ci}$  the centered variable i. e. the variable with its sample mean subtracted  $x_{ci} = x_i - \bar{x}_i$  for area  $i$  over  $N$  temporal samples. Graphs are then obtained :

- by finding a threshold for which the number of retained correlation values over the total number of possible connections represents the target density;
- by selecting areas as nodes and defining edges with correlation values over the retained threshold. The selection is made on significant correlations values (see also Appendix G).

The community detection algorithm is a greedy optimization of the modularity measure, from the default method of the `igraph` package [95], `igraph.Graph.community_fastgreedy` [96].

## Appendix C. Merging Tohoku and Waxholm atlases

Brain areas are obtained from the fusion of areas in the Tohoku atlas [24] and areas in the Waxholm Space atlas (WHS) [25]. The first contains cortical areas, the second focuses on sub-cortical ones. Nomenclatures from Tohoku atlas are: agranular insular area dorsal (AID), posterior (AIP), ventral (AIV); amygdalopiriform transition area (APir); primary auditory area (Au1); secondary auditory cortex, dorsal area (AUD); secondary auditory cortex, ventral area (AuV); cingulate cortex, area 1 (Cg1); cingulate cortex, area 2 (Cg2); dysgranular insular cortex

(DI); Entorhinal area (DIEnt); Entorhinal area (DLEnt); dorsolateral orbital cortex (DLO); entorhinal cortex (Ect); frontal association cortex (Fr3); granular insular cortex (GI); granular insular cortex dysgranular insular cortex (GIDI); lateral parietal association cortex (LPtA); primary motor cortex (M1); supplementary motor cortex (M2); entorhinal cortex, medial part (MEnt); medial parietal association cortex (MPtA); perirhinal cortex (PRh); Parietal association cortex (PtPC), (PtPD), (PtPR); retrosplenial cortex (RSD), (RSGb), (RSGc); primary somatosensory cortex (S1), barrel field (S1BF), dysgranular zone (S1DZ), dysgranular zone (S1DZ0), forelimb region (S1FL), hindlimb region (S1HL), jaw region (S1J), (S1Sh), trunk region (S1Tr), upper limb (S1ULp); second somatosensory cortex (S2); temporal cortex, association area (TeA); primary visual cortex (V1), binocular region (V1B), monocular region (V1M); secondary visual cortex, lateral part (V2L), mediolateral part (V2ML), mediomedial part (V2MM); Entorhinal area (VIEnt)

**Nomenclatures from WHS atlas are:** Clear Label (0); descending corticofugal pathways (cfp); substantia nigra (SN); subthalamic nucleus (STh); molecular layer of the cerebellum (Cb m); granule cell level of the cerebellum (Cb g); alveus of the hippocampus (alv); inferior cerebellar peduncle (icp); striatum (CPu); globus pallidus (GP); entopeduncular nucleus (EP); ventricular system (VS); medial lemniscus (ML); facial nerve (7n); anterior commissure, anterior part (aca); anterior commissure, posterior part (acp); ventral hippocampal commissure (vhc); thalamus (Th); septal region (Sep); optic nerve (2n); optic tract and optic chiasm (opt); pineal gland (Pi); inner ear (IE); commissure of the superior colliculus (csc); brainstem (BS); hypothalamic region (H); inferior colliculus (IC); superficial gray layer of the superior colliculus (SuG); periaqueductal gray (pag); fornix (f); mammillothalamic tract (mt); commissural stria terminalis (cst); deeper layers of the superior colliculus (DpG); periventricular gray (PVG); genu of the facial nerve (g7); pontine nuclei (Pn); fimbria of the hippocampus (fi); fasciculus retroflexus (fr); stria medullaris of the thalamus (sm); stria terminalis (st); posterior commissure (pc); glomerular layer of the accessory olfactory bulb (GIA); glomerular layer of the olfactory bulb (GI); olfactory bulb (OB); corpus callosum and associated subcortical white matter (cc); brachium of the superior colliculus (bsc); commissure of the inferior colliculus (cic); interpeduncular nucleus (IP); ascending fibers of the facial nerve (asc7); anterior commissure (ac); spinal trigeminal nucleus (SPN); spinal trigeminal tract (sp5); frontal association cortex (FrA); middle cerebellar peduncle (mcp); transverse fibers of the pons (tfp); habenular commissure (hbc); nucleus of the stria medullaris (SM); basal forebrain region (BF); supraoptic decussation (sox); bed nucleus of the stria terminalis (BST); pretectal region (PT); cornu ammonis 3 (CA3); dentate gyrus (DG); cornu ammonis 2 (CA2); cornu ammonis 1 (CA1); fasciola cinereum (FC); subiculum (S); postrhinal cortex (PrCx); presubiculum (PrS); parasubiculum (PaS); perirhinal area 35 (Per35); perirhinal area 36 (Per36); entorhinal cortex (Ent); lateral entorhinal cortex (LEnt); parabrachial nucleus (PB); Some areas are both atlases and are merged in the final one. Fusions of areas for left and right hemisphere are as follows, with the name of the resulting area given in Table 1, followed by the list of the regrouped areas: 0: {0}; ACC: {Cg1 r, Cg2 r, Cg1 l, Cg2 l}; Ins: {AID, AIP, AIV, DI, GI, GIDI}; APir: {APir}; AU: {AU1, AUD, AUV}; Ent: {DIEnt, DLEnt, Ect, MEnt, PRh, VIEnt, PrCx, Per35, Per36, Ent, LEnt}; Par: {LPtA, MPtA, PtPC, PtPD, PtPR}; M1: {M1}; M2: {M2}; RSC: {RSD, RSGb, RSGc}; S1: {S1, S1DZ, S1DZ, S1FL, S1HL, S1J, S1Sh, S1Tr, S1ULp}; S1BF: {S1BF}; S2: {S2}; TeA: {TeA}; V1: {V1, V1B, V1M}; V2: {V2L, V2ML, V2MM}; CPu: {CPu}; BG: {SN, STh, GP, EP}; Th: {Th}; Sep: {Sep}; H: {H}; DSC: {SuG, DpG, bsc}; pag: {pag}; BF: {BF}; PT: {PT}; HIP: {CA3, DG, CA2, CA1}; S: {S, PrS, PaS}; f: {cfp, MI, 7n, aca, acp, vhc, 2n, opt, csc, f, mt, cst, g7, fi, fr, sm, st, pc, cc, cic, asc7, ac, sp5, mcp, tfp, hbc, sox, FC, BST}; n: {IC, Cb m, Cb g, alv, icp, VS, Pi, IE, BS, PVG, Pn, GIA, GI, IP, SPN, SM, Fr3, FrA, DLO, OB, PB}; Note that 0 clear label, fibers (f) and not available areas (n) are not taken into account in the study.

Centers are projected on the left and right (LR) axis, inferior-superior (IS) axis and on the posterior-anterior (PA) with the origin at (0, 0, 0) given by the position of bregma at the apex of the most forward crossing fibers of the anterior commissure. Coordinates are given in millimeters. For sake of symmetry, the average left-right center of gravity is considered. The position of ACC is set to 0 on the LR axis, even if a small shift is present in the template. The smallest area TeA r has a volume of  $3.070 \text{ mm}^3$  (1572 voxels at  $0.002 \text{ mm}^3$  or 12 voxels at  $0.242 \text{ mm}^3$ ), the largest one CPu r, has a volume of  $45.143 \text{ mm}^3$  (23113 or 186 voxels, respectively).

## Appendix D. Extraction of signals from fMRI and atlas

The simple way to compute the signals in areas defined in the atlas is to transform the data from the fMRI low resolution volume to data in the high resolution volume where the atlas is

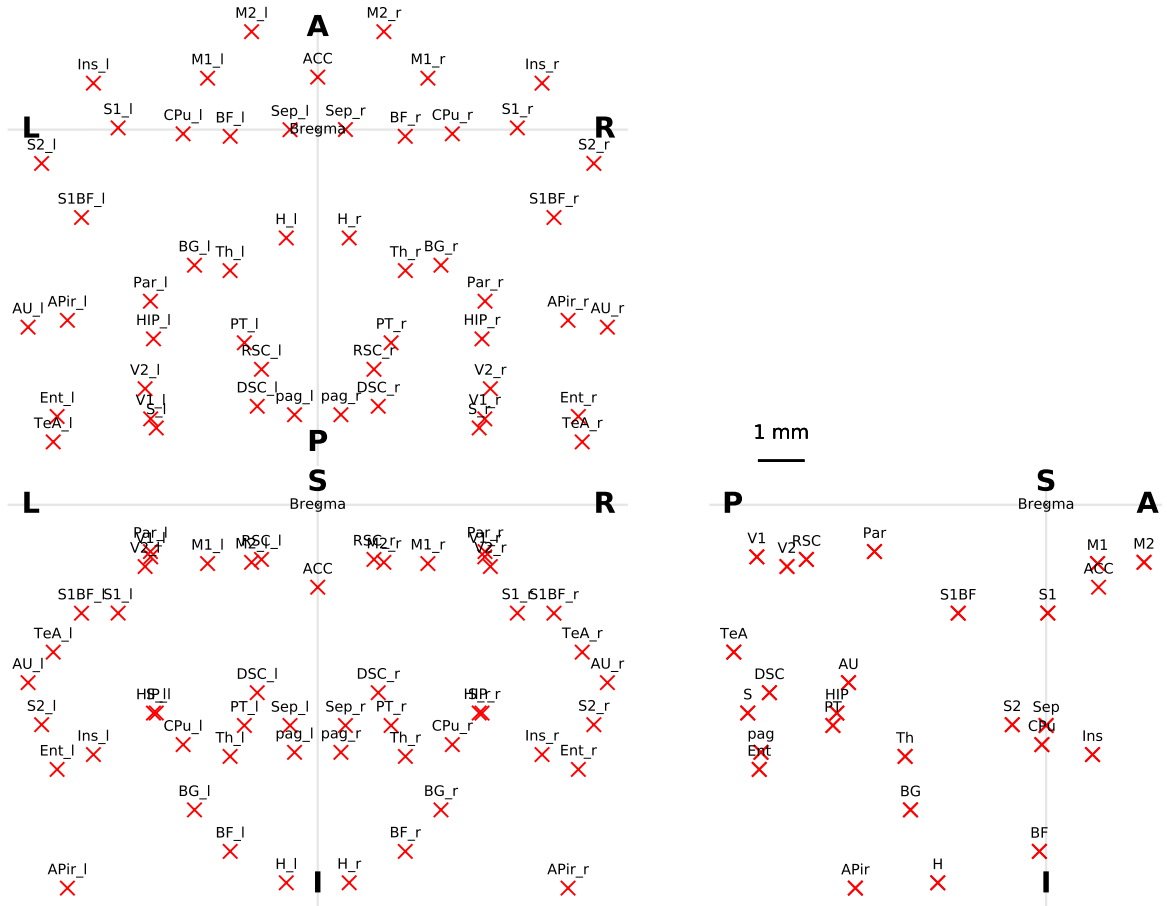


Figure C.9: Locations of the centers of gravity of the areas retained in this study on the three projections LR-IS, PA-IS, and PA-LR. Notations: left to right axis (LR), inferior to superior axis (IS), posterior to anterior axis (PA).

Table C.2: Brain areas in the atlas

#	name	label	x	y	z	N	h.	w. a.
0	<i>Clear Label</i>	0	0.2	-4.6	-4.5	1718587		
1	anterior cingulate cortex	ACC	0.2	-1.9	1.2	16670		acc
2	insular cortex right	Ins r	5.2	-5.7	0.9	11026	r	c
3	amygdalopiriform transition area right	APir r	5.7	-8.8	-4.3	3264	r	c
4	auditory cortex right	AU r	6.6	-4.2	-4.5	8781	r	c
5	entorhinal area right	Ent r	5.9	-6.1	-6.4	18392	r	c
6	parietal association cortex right	Par r	4.0	-1.2	-3.9	4557	r	c
7	primary motor cortex right	M1 r	2.7	-1.4	1.1	11027	r	c
8	supplementary motor cortex right	M2 r	1.7	-1.3	2.2	6619	r	c
9	retrosplenial cortex right	RSC r	1.5	-1.3	-5.5	8222	r	c
10	somatosensory 1 right	S1 r	4.6	-2.6	-0.0	20647	r	c
11	somatosensory 1 right, barrel field	S1BF r	5.5	-2.6	-2.0	9249	r	c
12	somatosensory 2 right	S2 r	6.2	-5.1	-0.8	2365	r	c
13	temporal cortex right, association area	TeA r	6.1	-3.5	-7.1	1572	r	c
14	primary visual cortex right	V1 r	4.0	-1.3	-6.5	9100	r	c
15	secondary visual cortex right	V2 r	4.1	-1.5	-5.9	4350	r	c
16	insular cortex left	Ins l	-5.0	-5.6	1.2	9002	l	c
17	amygdalopiriform transition area left	APir l	-5.7	-8.6	-4.3	3411	l	c
18	auditory cortex left	AU l	-6.5	-3.9	-4.5	9338	l	c
19	entorhinal area left	Ent l	-5.9	-5.8	-6.6	17859	l	c
20	parietal association cortex left	Par l	-3.6	-0.9	-3.9	4557	l	c
21	primary motor cortex left	M1 l	-2.3	-1.3	1.3	11160	l	c
22	supplementary motor cortex left	M2 l	-1.3	-1.3	2.2	5708	l	c
23	retrosplenial cortex left	RSC l	-1.1	-1.2	-5.3	7527	l	c
24	somatosensory 1 left	S1 l	-4.4	-2.3	0.1	21813	l	c
25	somatosensory 1 left, barrel field	S1BF l	-5.2	-2.3	-2.0	9578	l	c
26	somatosensory 2 left	S2 l	-6.3	-4.8	-0.8	2547	l	c
27	temporal cortex left, association area	TeA l	-5.9	-3.2	-7.1	1731	l	c
28	primary visual cortex left	V1 l	-3.6	-1.1	-6.6	9439	l	c
29	secondary visual cortex left	V2 l	-3.7	-1.3	-5.9	4276	l	c
30	caudate-putamen striatum right	CPu r	3.0	-5.6	-0.1	23113	r	s
31	basal ganglia right	BG r	2.6	-7.1	-3.1	3548	r	s
32	thalamus right	Th r	2.0	-5.8	-3.2	16544	r	s
33	septal region right	Sep r	0.6	-5.0	0.0	2167	r	s
34	hypothalamic region right	H r	0.6	-8.6	-2.5	2613	r	s
35	superior colliculus right	DSC r	1.4	-4.4	-6.3	6253	r	s
36	periaqueductal gray right	pag r	0.5	-5.5	-6.6	2745	r	s
37	basal forebrain region right	BF r	1.8	-8.0	-0.3	13759	r	s
38	pretectal region right	PT r	1.6	-5.1	-4.8	1805	r	s
39	hippocampus right	HIP r	3.7	-4.9	-4.7	19137	r	s
40	subiculum right	S r	3.6	-4.8	-6.8	5671	r	s
41	caudate-putamen striatum left	CPu l	-3.1	-5.2	-0.1	22803	l	s
42	basal ganglia left	BG l	-3.0	-6.7	-3.1	3238	l	s
43	thalamus left	Th l	-2.0	-5.6	-3.2	18218	l	s
44	septal region left	Sep l	-0.6	-5.0	-0.0	2733	l	s
45	hypothalamic region left	H l	-0.8	-8.5	-2.4	4246	l	s
46	superior colliculus left	DSC l	-1.3	-4.2	-6.3	6465	l	s
47	periaqueductal gray left	pag l	-0.5	-5.7	-6.3	3794	l	s
48	basal forebrain region left	BF l	-2.1	-7.7	-0.0	17694	l	s
49	pretectal region left	PT l	-1.7	-4.9	-4.8	1706	l	s
50	hippocampus left	HIP l	-3.8	-4.5	-4.8	18981	l	s
51	subiculum left	S l	-3.7	-4.6	-6.7	5962	l	s
52	<i>fasciculum</i>	<i>f</i>	-0.0	-4.8	-3.1	59834		
53	<i>not available</i>	<i>n</i>	-0.1	-5.8	-7.7	261557		

For each area: name of the area (name); label (label); the coordinates of the center of gravity (x, y, z), in mm, in the referential LR, IS, PA, with the origin centered at bregma location; N, the number of voxels in the area with a voxel size of 0.125 mm by 0.125 mm by 0.125 mm, in a cube of 136 by 102 by 180 voxels; the hemisphere (hemi.), left (l.) or right (r.); and the name of the wider area (w. a.), anterior cingulate cortex (acc), cortical (c) or subcortical (s). Rows in italic correspond to areas containing unlabeled subareas, undefined or unspecific residuals components not used in the study.

defined. The signal in one fMRI area is obtained as the sum of all voxels of this area in the atlas. This process implies to transform large voxels into small ones. The extraction of signals from the low resolution volume fMRI is possible if one can compute the proportion of each area contained in voxels of the fMRI. The processing of signals from the low resolution volume fMRI can then be seen as a weighted summation of voxel data for each area or a source separation problem. This enables to reduce the size of the processed images and reduce the computation times, while obtaining the same result as if working in the high resolution volume of the atlas. The mathematical framework for this operation is described here.

#### Appendix D.1. Equivalence of operations for processing low resolution volumes and high ones

Let  $V_1$  and  $V_2$  be two volumes containing MRI values at different resolutions.  $V_1$  and  $V_2$  contains respectively  $N_1$  and  $N_2$  voxels. We consider here that: each voxel  $i_1$  in  $V_1$  is associated to one label of one area of the atlas;  $V_1$  contains more voxels than  $V_2$ , or  $N_1 \geq N_2$ . This is generally the case when  $V_1$  is the anatomical volume and  $V_2$  is the fMRI volume with a lower resolution. Voxels in  $V_2$  intersect voxels in  $V_1$  at different locations. An example in two dimensions is given in Fig. D.10. Let  $K$  be the number of areas in the atlas and  $k$  the area label with index  $k$ . Let  $s(k, t)$  be the signal of area  $k$  at time  $t$ . Let  $s_1(k, t)$  and  $s_2(k, t)$  be the signals computed from  $V_1$  and  $V_2$ . The problem here is to obtain  $s_2(k, t) = s_1(k, t) = s(k, t)$ .

If the fMRI is obtained from  $V_2$ , values  $x_2(i_2, t)$  of voxels  $i_2$  are transformed into  $V_1$  such that  $i_1$  contains values  $x_1(i_1, t)$  by applying:

$$\begin{aligned} x_1(i_1, t) &= \sum_{i_2} \rho(i_1, i_2) x_2(i_2, t) \\ \text{or } X_1(t) &= R X_2(t) \quad \text{in matrix notation} \end{aligned} \tag{D.1}$$

where  $\rho(i_1, i_2)$  contains the fraction of  $i_2$  in  $i_1$ .  $s_1(k, t)$  can be computed by taking the voxels labeled with area  $k$ , or when taking  $\omega(i_1)$ , the label of the area related to the voxel  $i_1$  in the atlas, by:

$$\begin{aligned} s_1(k, t) &= \sum_{i_1 | \omega(i_1)=k} x_1(i_1, t) \\ \text{or } s_1(k, t) &= \sum_{i_1} w_1(k, i_1) x_1(i_1, t) \\ \text{or } S_1(t) &= W_1 X_1(t) \quad \text{in matrix notation} \end{aligned} \tag{D.2}$$

Where  $w_1$  is an indicator function giving  $w_1(k, i_1) = 1$  if  $\omega(i_1) = k$  and 0 otherwise. This leads to :

$$\begin{aligned} s_1(k, t) &= \sum_{i_1} w_1(k, i_1) \sum_{i_2} \rho(i_1, i_2) x_2(i_2, t) \\ S_1(t) &= W_1 R X_2(t) \end{aligned} \tag{D.3}$$

By taking  $w_2(k, i_2) = \sum_{i_1} w_1(k, i_1) \rho(i_1, i_2)$  or  $W_2 = W_1 R$  in matrix notation this leads to:

$$\begin{aligned} s_1(k, t) &= \sum_{i_2} w_2(k, i_2) x_2(i_2, t) \\ S_1(t) &= W_2 X_2(t) = S_2(t) \end{aligned} \tag{D.4}$$

This implies that  $s_1(t)$  computed in this way does not rely on the computation of  $x_1$  and can be noted  $s_2(t)$ .

Now, suppose that the signal recorded in  $V_2$ ,  $x_2(t)$  is a combination of  $s(k, t)$  such that:

$$X_2(t) = M S(t) \tag{D.5}$$

With  $M$  a mixing matrix of size  $(N_2, K)$ . The resolution of this equation is given by the pseudo inverse and leads to:

$$S(t) = (M^t M)^{-1} M^t X_2(t) \tag{D.6}$$

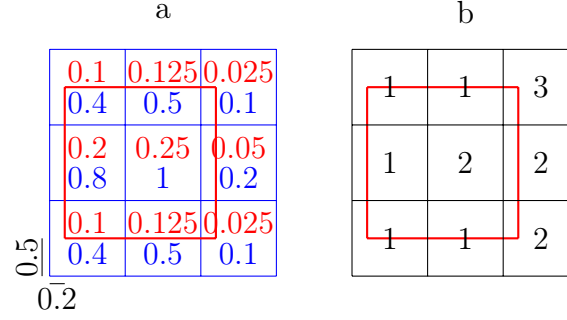


Figure D.10: Example of: a) the transformation from volume  $V_1$  to volume  $V_2$  and in blue the fraction  $\rho(i_1, i_2)$  and the proportion of components from  $s(i, t)$  in red (see text for details); b) the label contained in  $V_1$  and the intersection with  $V_2$ .

with  $^t$  the notation for the transpose operator. This implies that one admissible solution for  $S_2(t) = S(t)$  is obtained if  $W_2 = (\hat{M}^t \hat{M})^{-1} \hat{M}^t$  or  $W_2 \hat{M} = I$ .  $\hat{M}$  is then a pseudo inverse of  $W_2$  and can be chosen this way. Thus, the matrix  $W_2$  is obtained from  $R$  and  $W_1$  computed on our configuration, with the atlas in high resolution and the fMRI in low resolution. This last has been used to compute the signals of each area from the signals in the fMRI volumes in 3D.

#### Appendix D.2. Example of the equivalence of operations for processing low resolution volumes and high ones

An example of equivalence of operations for extracting brain areas signals from volume  $V_1$  (in blue) and  $V_2$  (in red) is given in Fig. D.10. In the 2D example, there are three labels  $\{1, 2, 3\}$ , nine voxels in  $V_1$  and one in  $V_2$ . If voxels are viewed rowwise, one can make the hypothesis that  $x_2$  is obtained from :

$$x_2(1, t) = M (s(1, t), s(2, t), s(3, t))^t$$

with  $M$  being a 3 by 1 matrix here. Taking the red proportions, obtained by computing the ratio of the areas of the intersections of the blue voxels over the area of the red voxel, and summing them for each labeled area leads to:

$$\begin{aligned} M &= (0.1 + 0.125 + 0.2 + 0.1 + 0.125, 0.25 + 0.05 + 0.025, 0.025) \\ M &= (0.65, 0.325, 0.025) \end{aligned} \quad (D.7)$$

The signal recorded in the red voxel is a composition of the signal in the three areas with a mixture according to  $M$ . In simple words, the signal recorded in the red voxel is made of 65 % of signal from area 1, 32.5 % of signal from area 2 and 2.5 % of signal from area 3.

All blue voxels are represented in a flat vector of dimension 9 in rowwise reading, from the top left to the bottom right. Taking the blue proportions, obtained by computing the ratio of the intersections of the red voxel with the areas of each blue voxel, gives  $R$ , the weights of voxels from  $V_2$  in  $V_1$ :

$$R = (0.4, 0.5, 0.1, 0.8, 1, 0.2, 0.4, 0.5, 0.1)^t$$

The mixture matrix in  $V_1$  from the 9 voxels to the 3 signals is given by  $W_1$ :

$$W_1 = \begin{pmatrix} 1 & 1 & 0 & 1 & 0 & 0 & 1 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

This leads to:

$$W_2 = W_1 R = (2.6, 1.3, 0.1)^t$$

961

$$\hat{M} \approx (0.31, 0.15, 0.01)$$

962  $\hat{M}$  and  $M$  are related with a factor equals to  $\sum_k \hat{m}_k \approx 0.47$ . Thus, signals at low resolution and  
 963 high resolution just differ by a scaling factor.

## 964 **Appendix E. Movement filtering with framewise displacement**

965 Following observations in [92], we examined carefully the potential influence of motions on the  
 966 obtained signals. In order to obtain the best estimates of FC during resting state, a selection of the  
 967 parts of signals not contaminated potentially by movement is performed. Samples with a framewise  
 968 displacement (FD) [92] over an experimental threshold are discarded from the study. The threshold  
 969 is set manually to 0.1 mm, based on observations, see infra., and corresponding to approximately  
 970 1/4 of the smaller voxel dimension (0.47 mm) and 1/9 of the larger voxel dimension (0.9 mm). All  
 971 samples following a sample with FD over this threshold are also marked as movement and discarded  
 972 for the study. This is done to take into account the effect of an hemodynamic response function  
 973 (HRF). The maximal duration of this HRF is set to 20 s based on values proposed in literature (see  
 974 [91] for example and references). The border effects of the signals at different scales are then taken  
 975 into account. To summarize, with technical terms of the field, we applied a scrubbing method with  
 976 boxcar filtering [37] taking into account HRF and wavelet support at different scales. An example  
 977 of the FD obtained for one rat is given in the Fig. E.11 a) with the impact on the signals at the  
 978 different frequency bands. An overview of the FD obtained for all rats is given in the Fig. E.11 b).  
 979 Three rats showed major displacements (FD over 0.47 mm). Distributions of FD are proposed in  
 980 the Fig. E.11 c-d) with the proposed threshold for this study. The impact of the selection of the  
 981 unretained samples on the signals at the different frequency bands is presented in the Fig. E.11  
 982 h-g) for all rats. The impact on signals at low frequencies (g - cD7) is important, as a consequence  
 983 of the width of the wavelet support at this scale, leading to few or no available samples to estimate  
 984 FC.

## 985 **Appendix F. Spectral analysis of fMRI signals and frequency bands**

986 To evaluate which part of the BOLD raw signal is filtered at each frequency band, the power  
 987 spectral densities (PSD) by groups are given in Fig. F.12. PSD are estimated using a Welch  
 988 averaging method (frequency precision 3.9e-3 Hz, duration of segments 128 s, overlap: 50%). PSD  
 989 per area and per rat are superimposed in black with a transparency factor. The median PSD  
 990 for each rat is computed and the average value is given in color for each group with a margin  
 991 corresponding to the standard error of the mean. An acquisition artifact, visible in all groups  
 992 including the dead animal, may be seen close to the cD2 upper frequency (0.5 Hz).

### 993 *Appendix F.1. Frequency bands used in other FC studies*

994 For comparison purpose, a graphical representation of the different frequency bands retained  
 995 in other rat studies is proposed in the Fig. F.13 (see also [36]).

## 996 **Appendix G. Construction of FC graphs from band filtered signals taking into account 997 significant correlations**

998 Two important points must be considered during the construction of FC graphs:



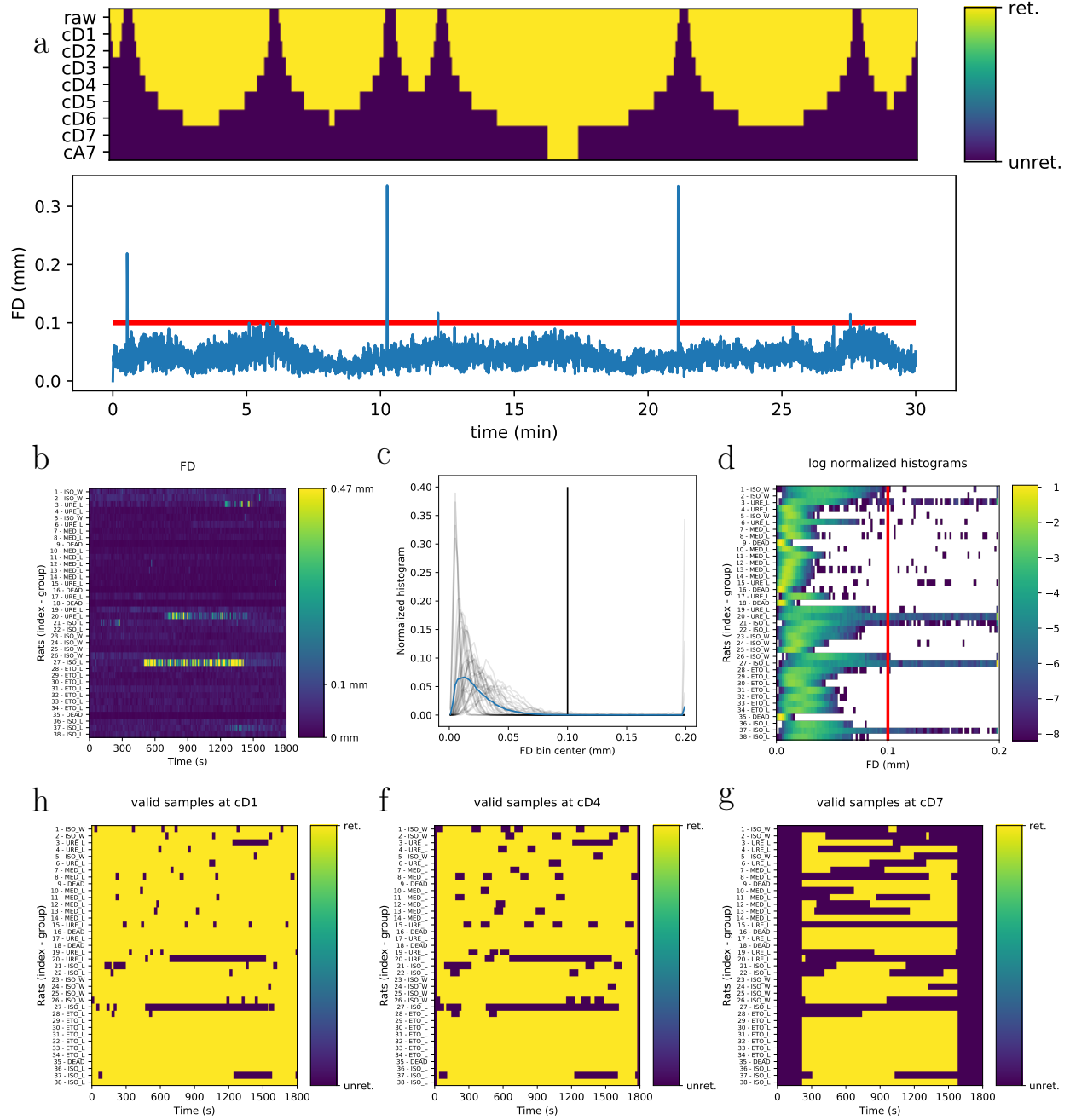


Figure E.11: Selection of retained rs-fMRI based on framewise displacement (FD). a) FD and impact on the raw signal and signals at different frequency bands. b) Overview of the FD for all rats. 0.47 mm represents the lowest dimension of voxels. 0.1 is the threshold used in the study. c) Normalized histograms of FD: rats histograms are proposed in gray. Values of FD over 0.2 are set to 0.2. Values in bins are normalized by dividing samples in bins with the total number of samples. The average histogram computed with all individuals is given in blue. d) Overview of histograms for each rat: a logarithmic version is proposed to enhance details and put in evidence that some values over the threshold are observed in several rats. e-h) Examples of the retained (ret.) and unretained (unret.) samples for the signal at cD1 (e), cD4 (f) and cD7 (g). The number of rejected samples increases as the frequency decreases because of the impact of the wavelet support.

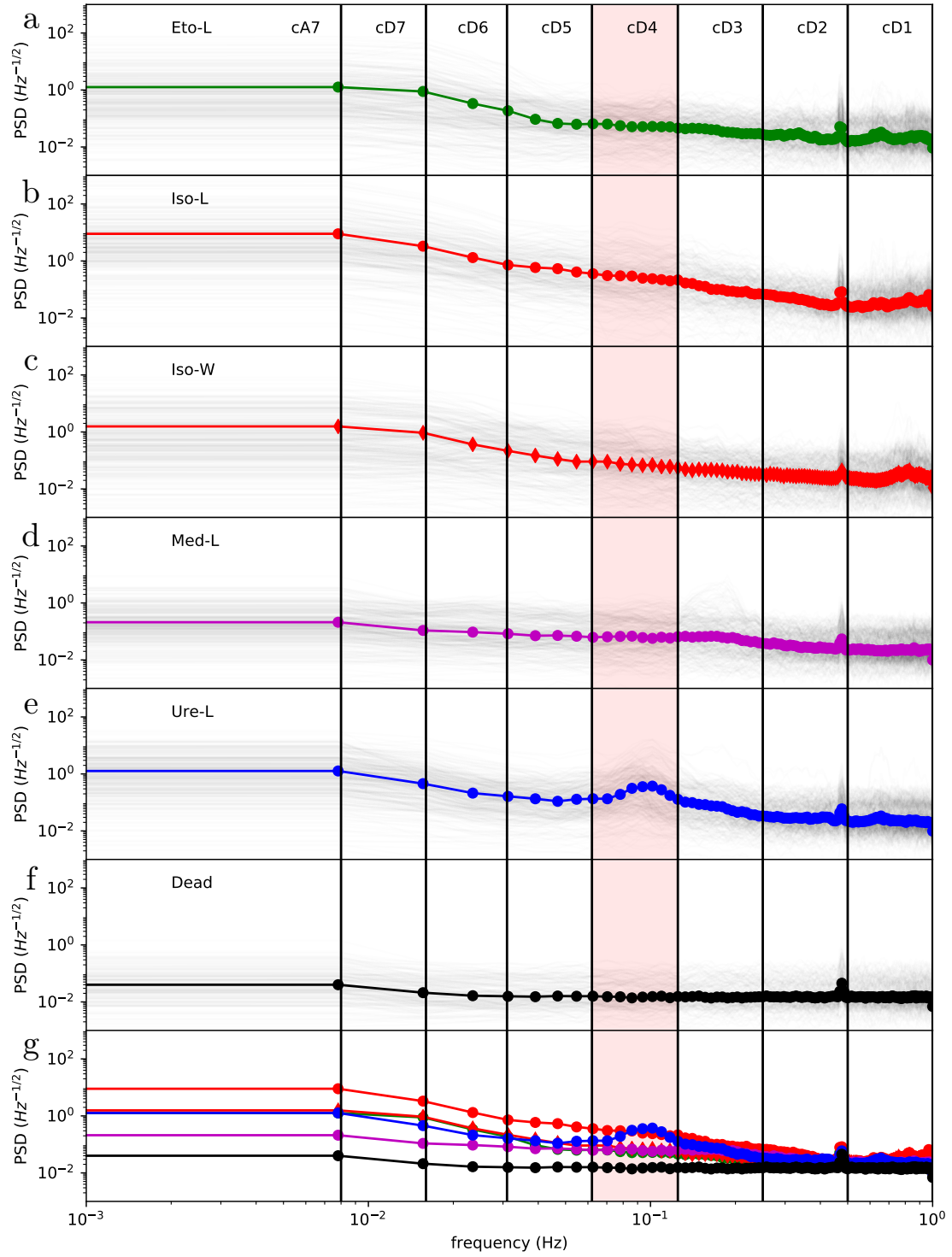


Figure F.12: Power spectral density (PSD) of BOLD raw signals by group. a-f) For each group, the 51 PSD of each animal are superimposed in black with a transparency factor. The median values per frequency is computed for each animal. Means and standard errors of the mean are given in colors. The cD4 band, retained in this study and called BOLD signal, is colored in red. g) Average PSD for the different groups.

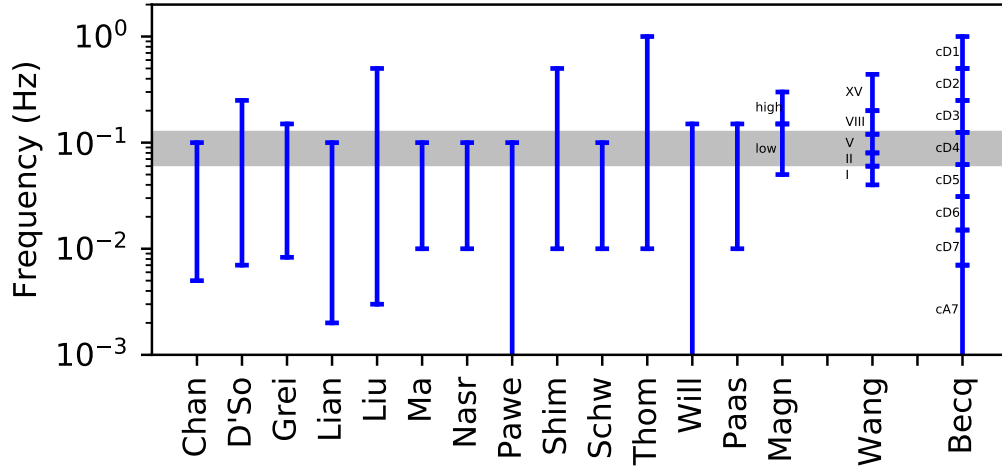


Figure F.13: Frequency bands retained in different rat rs-fMRI studies and all the frequency bands available in this study. The cD4 band (i.e. the BOLD signal) is filled in gray. Frequency cuts in Hz are given in parenthesis. The repetition time TR is also provided. [71] (0.005, 0.1) TR = 1.5 s; [40] (0.007, 0.25) TR = 2 s; [97] (0.0083, 0.15) TR = 2 s; [74] (0.002, 0.1) TR = 1 s; [44] (0.003, 0.5) TR = 0.612 s; [98] (0.01, 0.1) TR = 1 s; [58] (0.01, 0.1) TR = 2 s; [83] (0, 0.1) TR = 2 s; [68] (0.01, 0.5), and detrended to the second order, TR = 1 s; [85] (0.01, 0.1) TR = 1.7 s; [16] (0.01, 1), and linear detrend, TR = 0.5 s; [14] (0, 0.15), and normalisation, TR = 0.1 s; [6] (0.01, 0.15), TR = 2 s; [76] (0.05, 0.15, 0.30) and normalisation TR = 0.5 s; [99] (0.04, 0.06, 0.08, 0.12, 0.2, 0.44) simulated TR = 2 s; Becq et al., this study, (0, 0.007, 0.015, 0.031, 0.062, 0.125, 0.25, 0.5, 1) TR = 0.5 s.

- Spurious correlations depend of the number of samples used to compute correlations. This number is function of the frequency band of interest. For a lower frequency of interest, fewer samples are needed, and spurious correlations are less likely.
- Spurious correlations are obtained when comparing multiple values of correlations as a consequence of multiple connections between brain areas. This implies to adjust level for multiple corrections.

First, we evaluate whether there are enough signals in the graphs to get a sufficient number of edges by counting the number of significant edges different from zero. This number is computed using the results from [26] where it is shown that the Fisher transform  $z$  of the empirical correlation  $\hat{\rho}$  follows a Gaussian law with mean the Fisher transform of the true value and variance depending on the number of available wavelet coefficients at scale  $j$ , denoted  $N_j$  here. The confidence interval  $[\rho_m, \rho_M]$  for the correlation of two random signals is given in [100, 26] at a given scale  $j$  with a Daubechies wavelet filter of length  $L$ .

$$\rho_{m,M} = \tanh \left( h(\rho) \pm \frac{\Phi^{-1}(1-p)}{\sqrt{(\hat{N}_j - 3)}} \right) \quad (\text{G.1})$$

$$\hat{N}_j = \frac{N}{2^j} - [L_j] \quad (\text{G.2})$$

$$L_j = (L - 2)(1 - 2^{-j}) \quad (\text{G.3})$$

where  $\hat{N}_j$  is the number of wavelet coefficients associated with scale  $\lambda_j$ ,  $h(\rho) = \tanh^{-1}(\rho)$  and  $\Phi^{-1}(p)$  is the  $p$ th quantile of the standard normal distribution. A Bonferroni procedure is applied

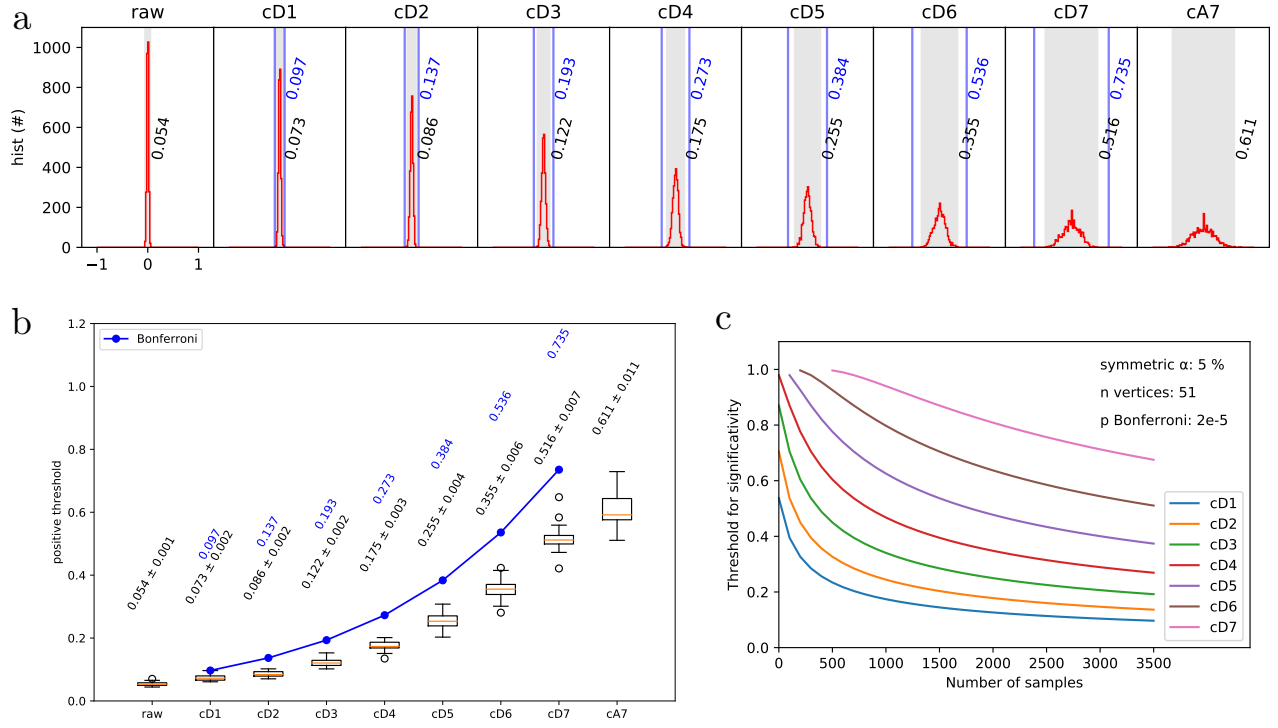


Figure G.14: Distributions of correlations at different scales for one drawing of random signals and the empirical upper limit threshold of the segment containing 99.998% (95% Whitcher + Bonferroni procedure) of the values. a) Histograms estimated on 100 bins between  $[-1, 1]$ ; b) Boxplot of the thresholds containing 99.998% of the distributions at different wavelet levels. Values from the Bonferroni procedure with a an initial symmetrical confidence interval at 95 % are in blue. Values from empirical distributions of the simulated noises are given in black. c) Threshold for significant correlations in function of the number of samples per frequency bands. Plots are obtained with Eq.G.4 setting  $p=2e-5$  for taking into account Bonferroni corrections on a symmetric test at level  $\alpha = 5\%$  with 51 areas.

to the  $p$  value to take into account  $ne = nv(nv - 1)/2 = 51 \times 50/2 = 1275$  repetitions of the  $nv = 51$  brain areas to obtain a level with  $p_{bf} = p/ne$ .

A model of uncorrelated random noises, with  $\rho = 0$ , is used to obtain significant thresholds at an initial symmetrical confidence interval set to 95 % leading, after Bonferroni correction, to a level  $p_{bf} = 0.025/1275 = 1.96e-5 = 0.002\%$ . 51 signals with 3600 samples with i.i.d. samples following a Gaussian distribution  $N(0, 1)$  are simulated. The same wavelet decomposition than the one used for the BOLD signals is applied and the  $ne$  correlations are estimated for each wavelet coefficients. Examples of distributions of the correlations at different wavelet levels are given in Fig. G.14a. Values are ranked and the correlation value of the  $i_{th} = ne(1 - p_{bf}) = 1275 \times (1 - 1.96e-5) = 1275$  th (max) is retained as the threshold level for which 99.998% of  $abs(C)$  is contained. Theoretical values given by Whitcher and the Bonferroni procedure are given in blue. Distributions and values of these thresholds at different wavelet levels are given in Fig. G.14b with the segment containing 99.998% of the values represented in gray.

Differences between theoretical thresholds and experimental ones increase with scale indices. Unfortunately, there is no result about the theoretical distribution of the correlation coefficients to the best of our knowledge and it seems not easy to obtain.

Because of the scrubbing based on FD, signals obtained on different rats do not have the same

size. Besides, nonsuccessive samples are retained and in this condition, Eq. G.1 can not be applied properly. But since the retained samples are already taking into account the influence of the wavelet support, the following formula is used to obtain the limits of significant correlations:

$$\rho_{m,M} = \tanh \left( h(\rho) \pm \frac{\Phi^{-1}(1-p)}{\sqrt{\left(\frac{N_r}{2^j} - 3\right)}} \right) \quad (\text{G.4})$$

with  $N_r$  the number of retained samples. The limits of significant correlations at different scales in function of the number of retained samples is proposed in Fig. G.14c putting in evidence the importance of the number of samples for obtaining significant correlations at low frequencies (cD7).

This allows the computation of a confidence interval to remove the correlations that are below the significance level. For examples, the theoretical Bonferroni threshold is 0.273 for the cD4 band, if all samples are retained with  $N = 3600$  for Eq. G.1 and  $N_r = 3495$  for Eq. G.4 with a Daubechies wavelet of size  $L=8$ . This is illustrated on the dead rats data where no signal is detected in the graphs. Following this first step, the idea to use graphs is to be able to compare them. The comparison of graphs with different number of edges is difficult because graph metrics are directly impacted by this factor. We choose here to force the graphs to have the same number of edges, selected from the significant ones. Because dead rats have a too small number of significant edges, it is not possible to construct a graph with a constant number of edges for these animals.

## Appendix H. Supplementary Analyzes

### Appendix H.1. Scatter plots

Scatter plots of SV, baseline CBF, BOLD variation and  $\langle C \rangle$  BOLD-BOLD (Fig. I.20 to Fig. I.28) can be further analyzed here. Most of the individuals in each group remain close to each other indicating an homeostatic state specific to each anesthetic. The effect of the strain can be evaluated on the Iso-L and Iso-W groups that show separated clouds. There are some outliers: One individual with Temp  $< 35$  °C in the Eto group but this is caused by problems with the temperature sensors (regular values between 35 and 37 °C); One individual with SpO2  $< 80\%$  in the Ure group. Estimation of regression parameters and correlation coefficients are given in each figure, with outliers discarded for the regression. There are some statistically significant correlations:

- weak correlations ( $|R| < 0.5$ ): BOLD variation with (RR, Temp, Error rate, raw BOLD);  $\langle C \rangle$  BOLD-BOLD with (HR, Error rate, raw BOLD); HR with Error rate; RR with SpO2; Temp with Error rate; Weight with raw BOLD.
- medium correlations ( $0.5 \leq |R| < 0.75$ ): BOLD variation with ( $\langle C \rangle$  BOLD-BOLD, Error rate);  $\langle C \rangle$  BOLD-BOLD with Temp; baseline CBF with (HR, Temp); HR with Temp; RR with Error rate; SpO2 with Error rate.
- one strong correlation ( $|R| \geq 0.75$ ): BOLD variation with  $\langle C \rangle$  BOLD-BOLD.

The error rate is correlated with several variables. This can be explained by the fact that when the signal is lost on the oximeter, either the animal is not stable during the anesthesia creating some artifacts measures, either the animal is in a state in which he needs to regulate himself. If the animal is suffering, reactions can induce error rates, and these reactions are observed and can be

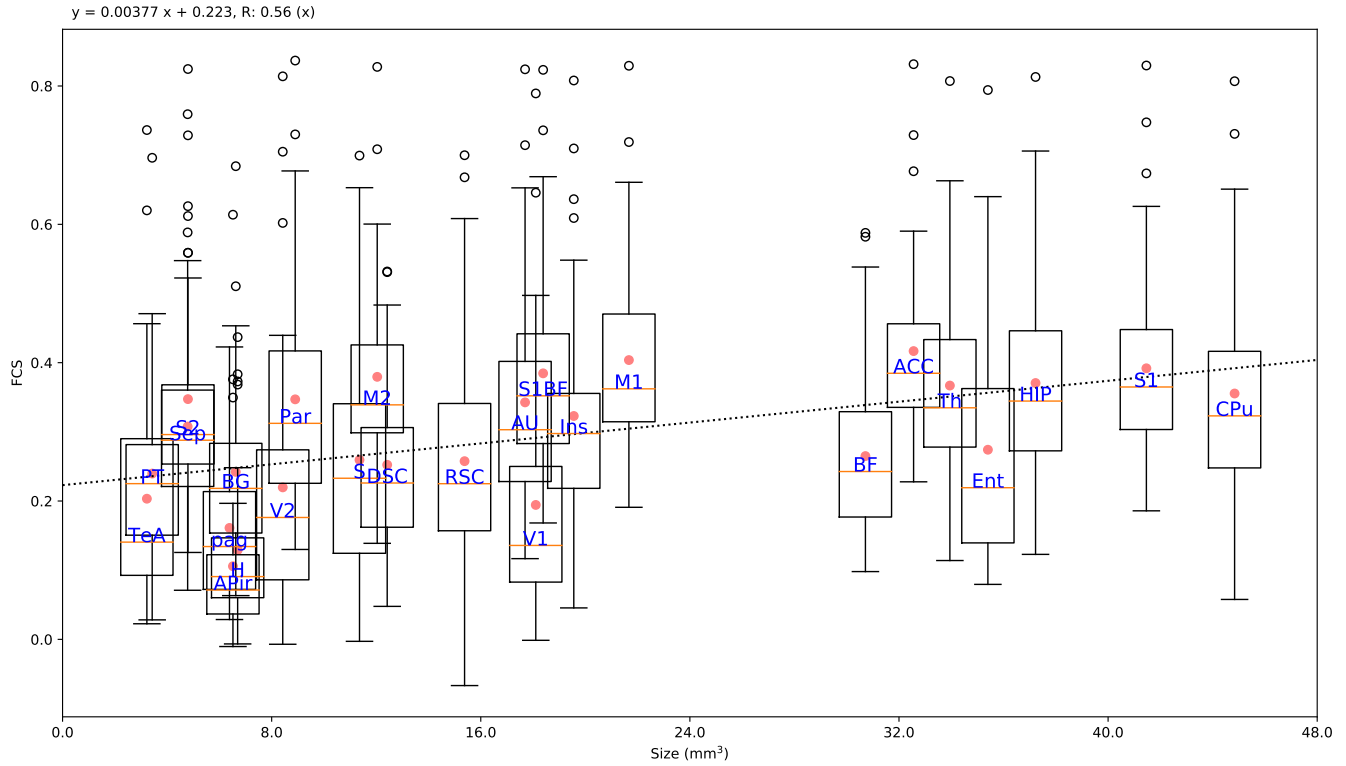


Figure H.15: FCS distributions across rats versus area size. Each box corresponds to one area with left and right areas pooled together. The dotted line corresponds to a linear fit estimated from the average values of the areas represented with red dots.

related to the activity in BOLD signals. These reactions can also be related to an increase in  $\langle C \rangle$  BOLD-BOLD with answers to the same conditional stimuli, e.g. pain, suffering, wake up. The temperature is related to different variables especially, HR, baseline CBF, BOLD variation and  $\langle C \rangle$  BOLD-BOLD. This potentially indicates that 'hot rats' are better perfused, have stronger neural activities and stronger FCS between areas. BOLD variation has a strong correlation with  $\langle C \rangle$  BOLD-BOLD indicating that rats with high BOLD variations show high correlations values even after taking into account the motions correctly.

## Appendix H.2. Influence of area size on FCS

Mean FCS across animals as a function of area size are shown Fig. H.15. There is a medium correlation ( $R=0.56$ ,  $p<0.01$ ) between the area size and the FCS. The slope between these two variables is weak (0.0035 per  $mm^3$ ) and the high variability of the distributions indicates a low influence of the size of the area on FCS.

## Appendix H.3. Influence of distances on pairwise correlations

The distributions of pairwise correlations in function of pairwise distances between areas are proposed in Fig.H.16 for each rat in each group (top) and for all rats in groups (bottom). Significant correlations are values above thresholds represented with black lines. The distributions are homogeneous within groups except for some individuals, in particular in the Iso groups. Values of correlations above thresholds are observed for all pairwise distances, even at long distances, except for dead rats were only few spurious values are observed at short distances. This indicates that long range correlations in all groups are observed.

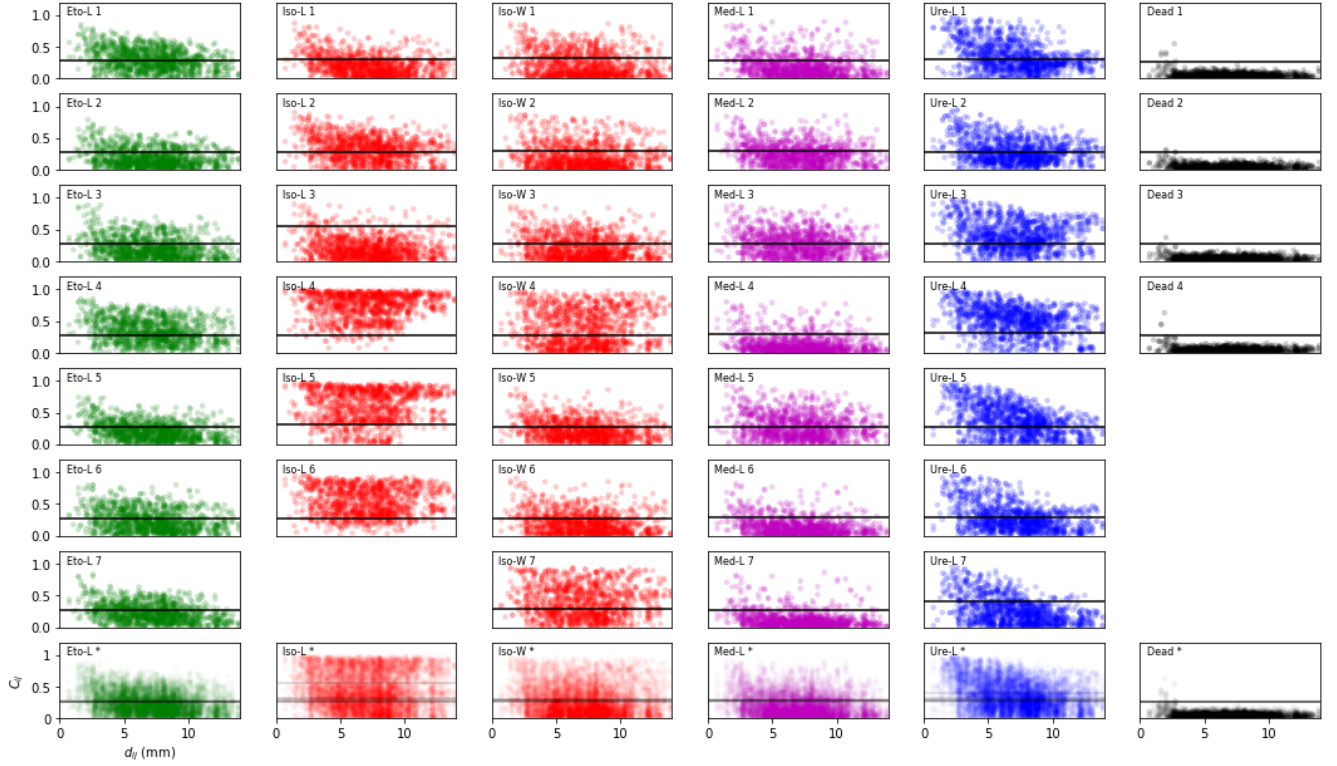


Figure H.16: Correlations between pairs of areas in function of distances between them. Scatters are given for each rat in each group and the total distribution is given in the bottom row indicated by \*.

#### 1090 Appendix H.4. Influence of distances on pairwise FCS

1091 The distributions of FCS computed on significant correlations, versus the spatial locations of  
 1092 areas are given in Fig.H.17 for the different axis (LR, IS, AP) and in function of the Euclidean  
 1093 distances to the Bregma origin. An area is represented by the coordinates of its center of gravity.  
 1094 Anesthetized rats are pooled together to compute the box and whisker plots. Linear regressions  
 1095 between the median values of FCS and distances are also computed. FCS does not seem to be  
 1096 influenced by medio-lateral positions, even near ears (AU areas). There is a decrease of FCS in  
 1097 function of the position to the sensors put in evidence in the dorso-ventral (IS) representation.  
 1098 There is a decrease of FCS in function of the antero-posterior localization of the areas. These  
 1099 attenuations of FCS are put in evidence with the representation in function of the Euclidean  
 1100 distance to Bregma with a medium significant linear correlation ( $R=-0.68$ ,  $p=3.2e-8$ ), a light slope  
 1101 ( $-0.02$  / mm), and a high variability of distributions for some areas between rats. For all these  
 1102 representations, range of values are more or less extended in function of areas.

#### 1103 Appendix H.5. Average matrices per group

1104 Average matrices per group of the significant connections are computed. Representations using  
 1105 graphs with nodes represented by the center of gravity of each area, on the 3 projections LR, IF,  
 1106 AP, are proposed in Fig. I.38a. Edges are plotted one over the other with the lowest values plotted  
 1107 first and the highest ones last. Different colors are also retained to show highest connected areas.  
 1108 Highest connections are more or less different from one group to another. All areas are connected  
 1109 on the anesthetized groups except one for Eto-L. Few edges are observed on the Dead group and  
 1110 correspond to edges between close areas in the anatomic template. The average matrices obtained



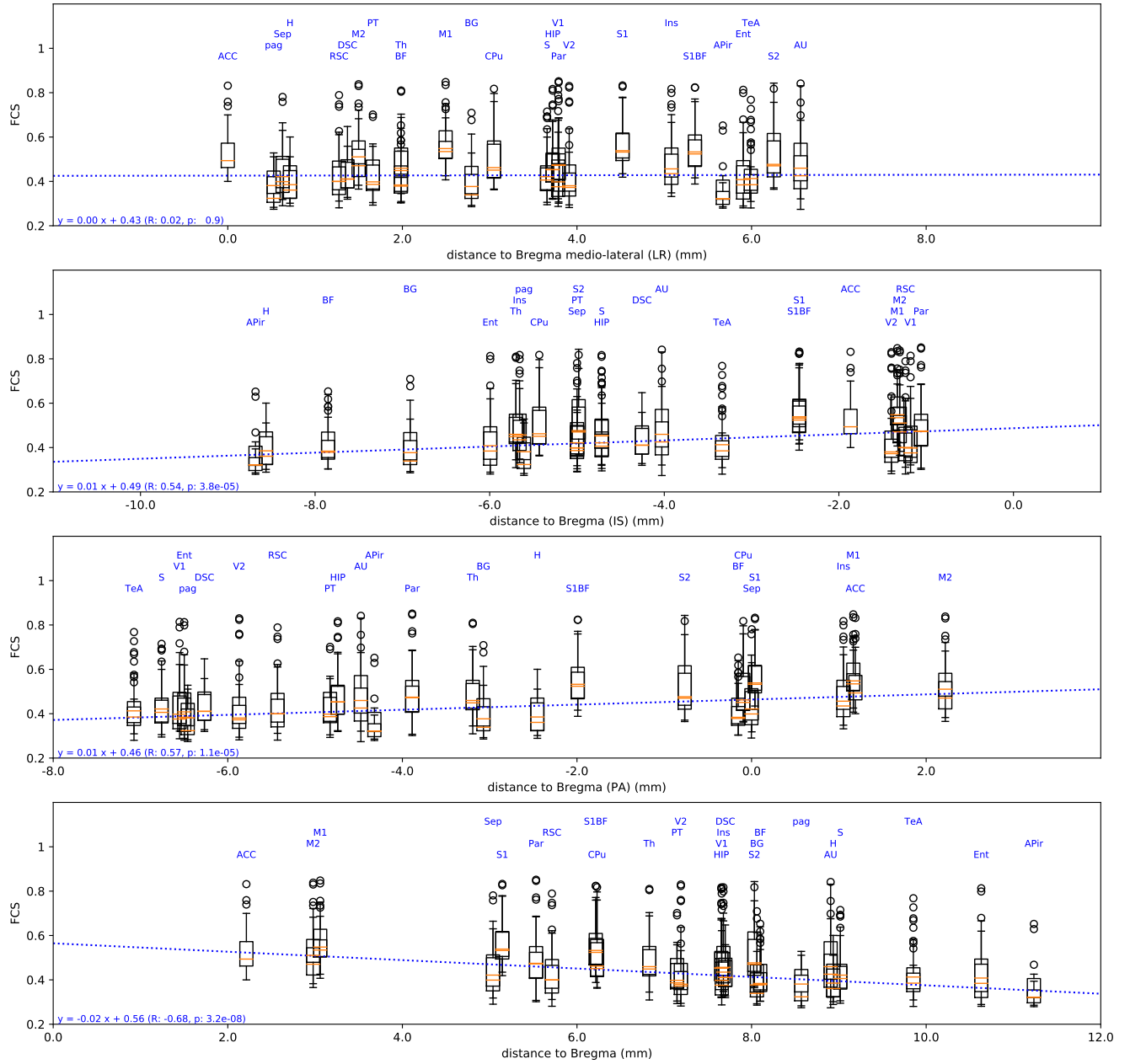





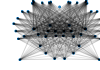


Figure H.17: Distribution of FCS versus spatial position of areas. The origin Bregma is at (0, 0, 0) in the LR, IS, AP coordinate system. A linear regression is fitted with median values of FCS. a) For medio-lateral positions (LR axis). b) For dorso-ventral positions (IS axis). c) For antero-posterior positions (PA axis). d) For Euclidean distances to Bregma.



Table H.3: Distributions of connections per group.

	ACC 	inter 	intra 	c - c 	s - s 	c - s 
$\rho = 0.05$						
Eto-L	16 $\pm$ 0	27 $\pm$ 2	55 $\pm$ 1	48 $\pm$ 3	17 $\pm$ 3	17 $\pm$ 2
Iso-L	11 $\pm$ 1	40 $\pm$ 1	48 $\pm$ 2	73 $\pm$ 4	11 $\pm$ 5	2 $\pm$ 1
Iso-W	19 $\pm$ 0	42 $\pm$ 1	38 $\pm$ 1	65 $\pm$ 3	9 $\pm$ 3	4 $\pm$ 1
Med-L	13 $\pm$ 1	41 $\pm$ 1	44 $\pm$ 1	73 $\pm$ 3	10 $\pm$ 2	2 $\pm$ 0
Ure-L	2 $\pm$ 0	1 $\pm$ 0	96 $\pm$ 1	56 $\pm$ 4	29 $\pm$ 4	11 $\pm$ 3
$\rho = 0.1$						
Eto-L	13 $\pm$ 0	34 $\pm$ 2	51 $\pm$ 1	40 $\pm$ 1	17 $\pm$ 2	27 $\pm$ 1
Iso-L	9 $\pm$ 0	42 $\pm$ 1	48 $\pm$ 2	66 $\pm$ 5	15 $\pm$ 5	8 $\pm$ 1
Iso-W	14 $\pm$ 0	43 $\pm$ 1	42 $\pm$ 1	56 $\pm$ 2	13 $\pm$ 4	16 $\pm$ 2
Med-L	12 $\pm$ 0	41 $\pm$ 0	46 $\pm$ 0	61 $\pm$ 3	16 $\pm$ 3	10 $\pm$ 1
Ure-L	3 $\pm$ 0	4 $\pm$ 1	92 $\pm$ 2	45 $\pm$ 5	31 $\pm$ 4	19 $\pm$ 2
Full	4	49	47	30	18	48

Mean  $\pm$  SEM are given in %. Notations: interhemispheric (inter), intrahemispheric (intra), cortex (c), subcortical (s)

with at least significant values from 4 animals are also represented as graphs in Fig. I.38b. Connections are enhanced, with enhanced differences between groups. No connections are persistent in the Dead group. The number of significant values obtained on each connections is displayed in Fig. I.38c. where a probability of 1 indicates that all rats of the group have this connection significant. Stable networks within groups are enhanced.

The observations that can be made on average graphs across group Fig. I.38 may also be observed on individual graphs (Fig. I.32 to Fig. I.37):

- For Eto-L, there is a widespread fronto-parietal complex associating frontal and inter-hemispheric sensory communities, with shared connections between left and right cortical areas. The connection with a large subcortical area is preserved with an important role of the thalamus.
- For Iso-L, regarding cortical areas, there are widespread fronto-parietal, ipsi- and inter-hemispheric communities containing the motor area in one hemisphere connected to its contralateral sensory area. There are wide subcortical communities associated to cortical communities. The cortical-subcortical connections mediated by Th and HIP are part of the highly correlated coefficients, and indicate connections with visual areas.
- For Iso-W, only four communities are put in evidence: a widespread cortical, well-connected module; an isolated subcortical interhemispheric Th-HIP; An ACC-CPu module well connected to cortical areas and an Ins module, more or less present in individuals.
- For Med-L, there is a widespread inter-hemispheric fronto-parietal community connected to an inter-hemispheric sensory module connected to HIP and RSC. The subcortical inter-hemispheric communities is weakly connected to cortical areas.
- For Ure-L, large left and right cortical fronto-parietal communities are shown. These two cortical left-right communities are weakly connected. Two subcortical left-right communities are also disconnected. There are few connections between these cortical-subcortical communities. The connections between cortical-subcortical communities, left and right communities are weak. The role of ACC seems weaker than in other groups.

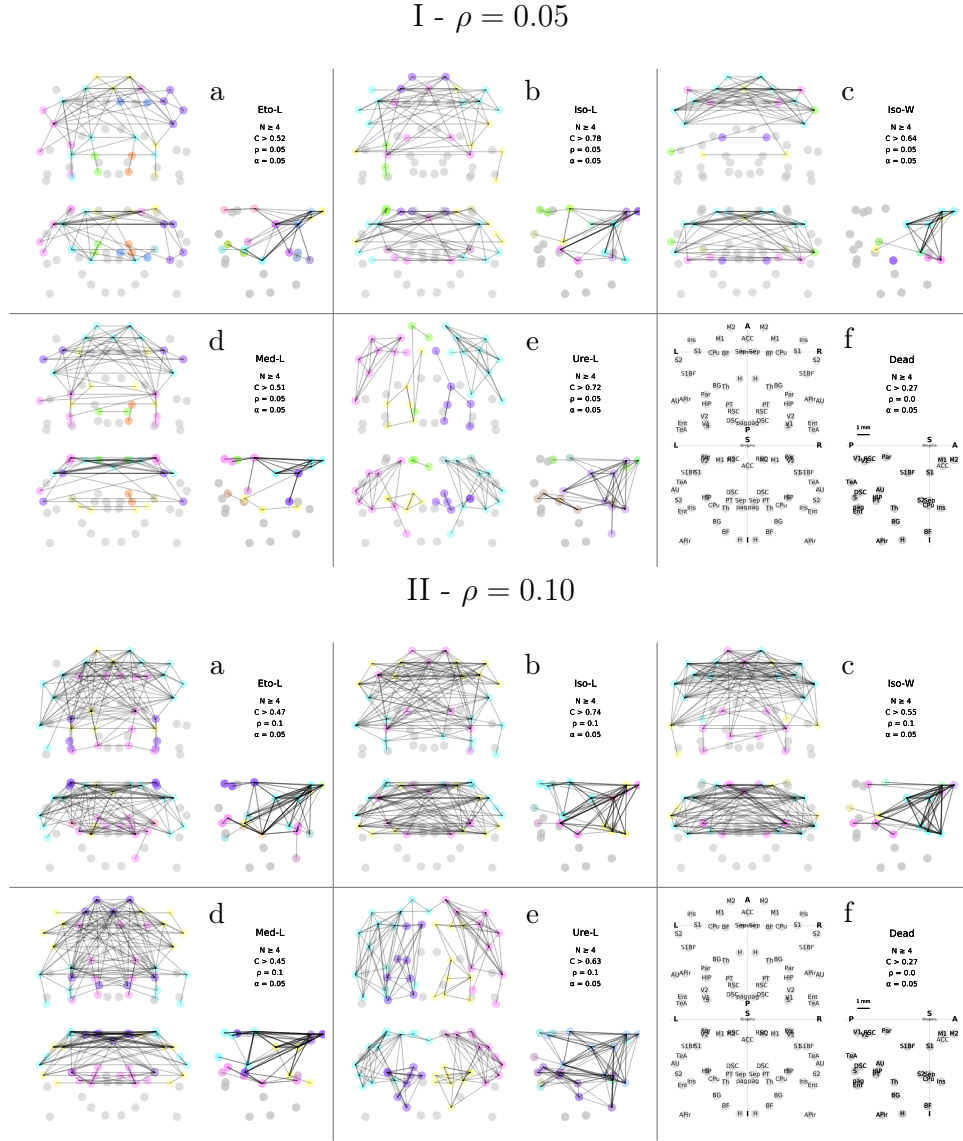


Figure H.18: Graphs based on the average correlation matrices for each group at two density: I) by taking the 63 most significant correlated values (density = 0.05) with at least 4 animals; II) by taking the 127 most significant correlated values (density = 0.1) with at least 4 animals.

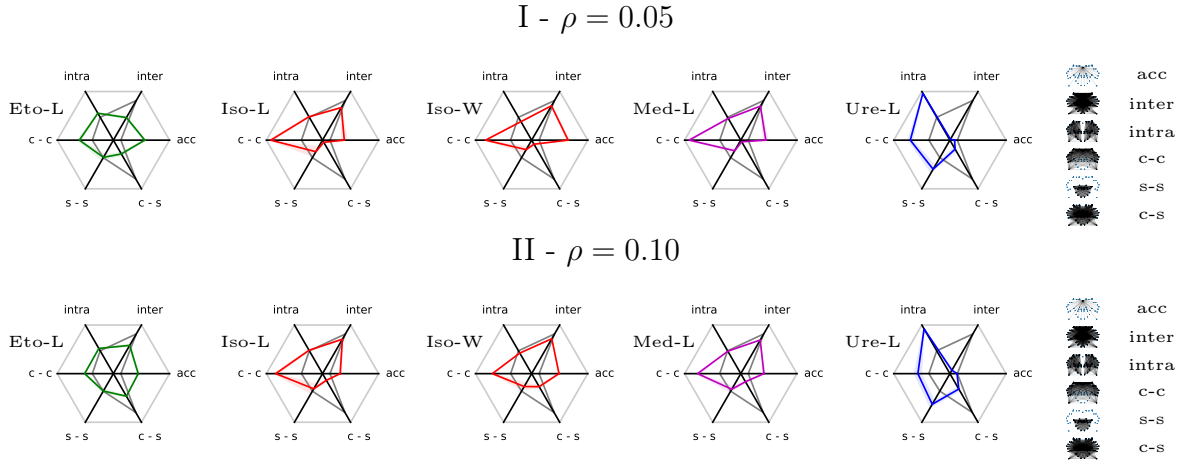


Figure H.19: Radar plots for the distributions of connections for a)  $\rho = 0.05$  and b)  $\rho = 0.1$ . The gray curve corresponds to the fully connected graph (Full).

## Appendix H.6. Comparison of graphs at different densities

Distributions of connections per wide areas are given in Table H.3 and Fig. H.19 for the two densities  $\rho = 0.05$  and  $\rho = 0.1$ . The comparison of values between densities indicates that from the low density graphs at  $\rho = 0.05$  to the dense one at  $\rho = 0.1$ , the ratio of c-s connections increased for all groups, especially for Med-L and Iso-W, towards the ratio of the complete graph. For these groups, the c-c balance decreases and the s-s balance increases. This is also observed on the global patterns observed in Fig. H.18 for  $\rho = 0.05$  and  $\rho = 0.1$ . In vivo graphs differ from the theoretical full-graph, which has a small ACC contribution (4%), and equivalent inter- and intra-hemispheric connections. In vivo, the contribution of ACC is comparable across groups (between 9 and 14 %), except for Ure-L for which it barely contributes (3 %) (values given for the density  $\rho = 0.1$  but same comparisons can be drawn from  $\rho = 0.05$ ). Ure-L also differs by the fact that most connections (92 %) are intra-hemispheric rather than inter-hemispheric (4 %). For the other groups, the balance between inter-hemispheric and intra-hemispheric proportions are comparable with less intra-hemispheric connections for the Eto-L group. For this group, the cortical-subcortical proportion is the highest one (27 %), to the detriment of the cortical-cortical proportion (the lowest one). Iso-L shows the lowest proportion of subcortical-cortical connections (8 %) and the highest proportion of cortical cortical connections (66 %) and is comparable with Iso-W and Med-L. A graphical comparison using radar profiles is proposed in Fig. H.19, that highlights the similarities and dissimilarities between groups.

## Appendix I. Supplementary figures

### Appendix I.1. 1

### Appendix I.2. 2

### Appendix I.3. 3

### Appendix I.4. 4

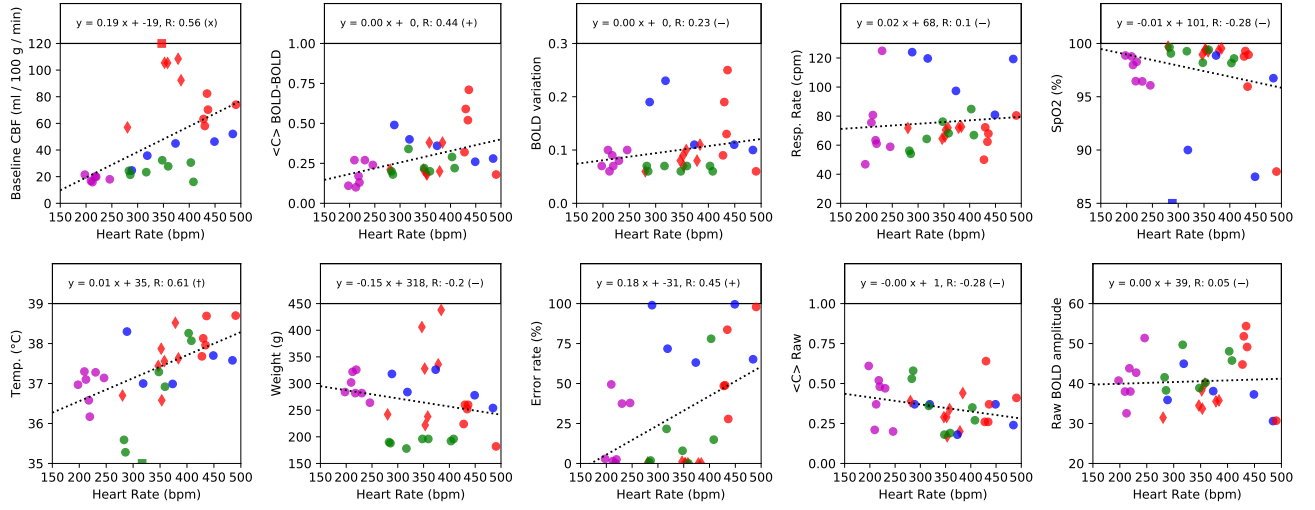


Figure I.20: Distributions of variables per group with scatter plots as a function of HR. Outliers are marked with a square. A linear trend is computed without the outliers.

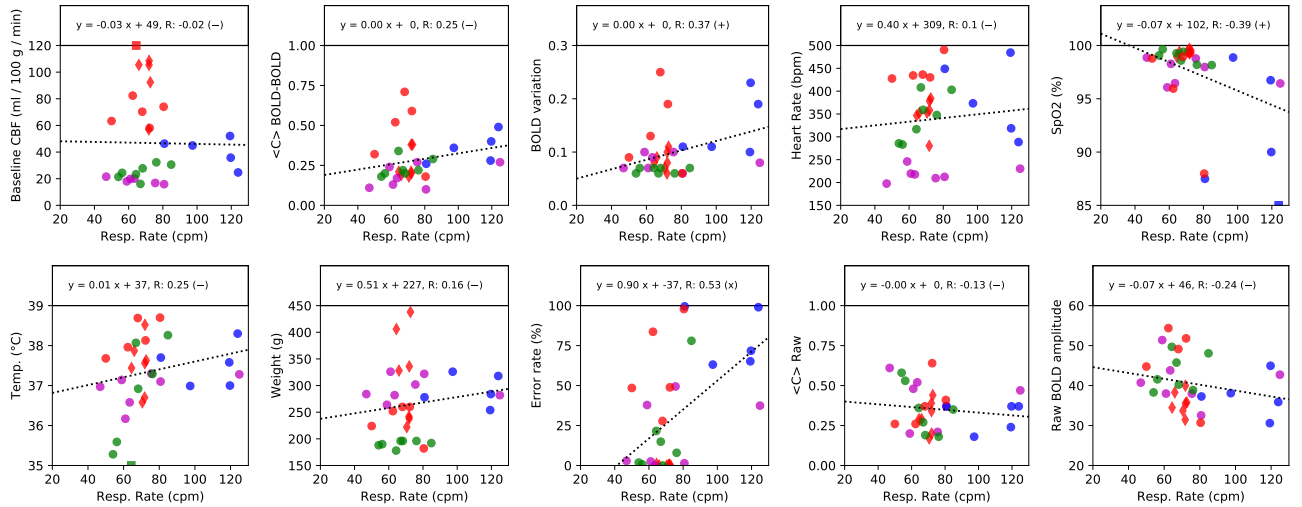


Figure I.21: Distributions — scatter plots — as a function of RR.

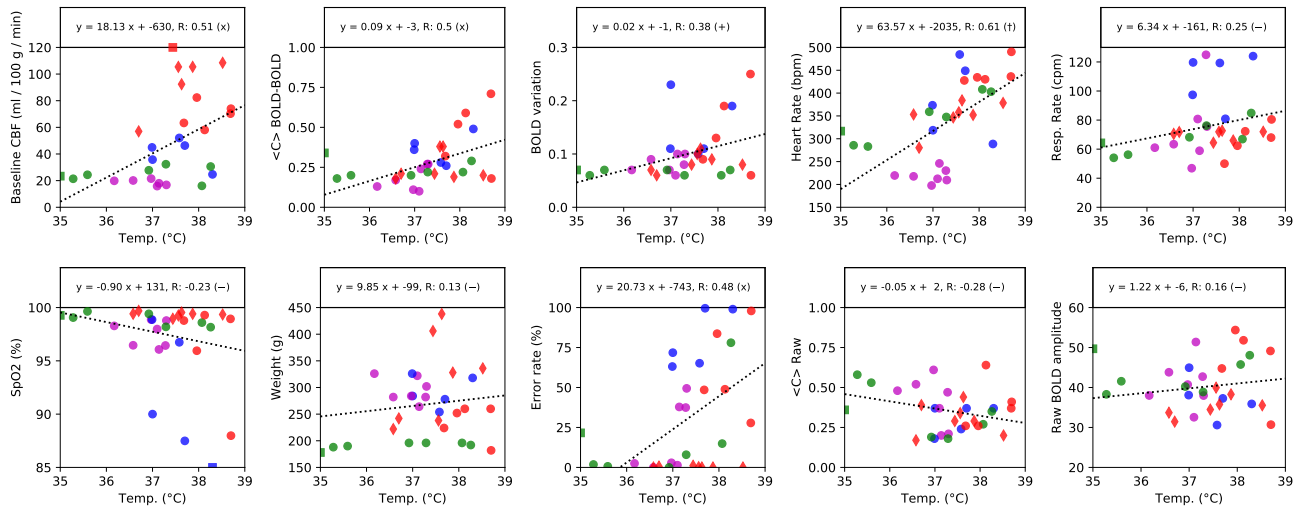


Figure I.22: Distributions — scatter plots — as a function of Temp.

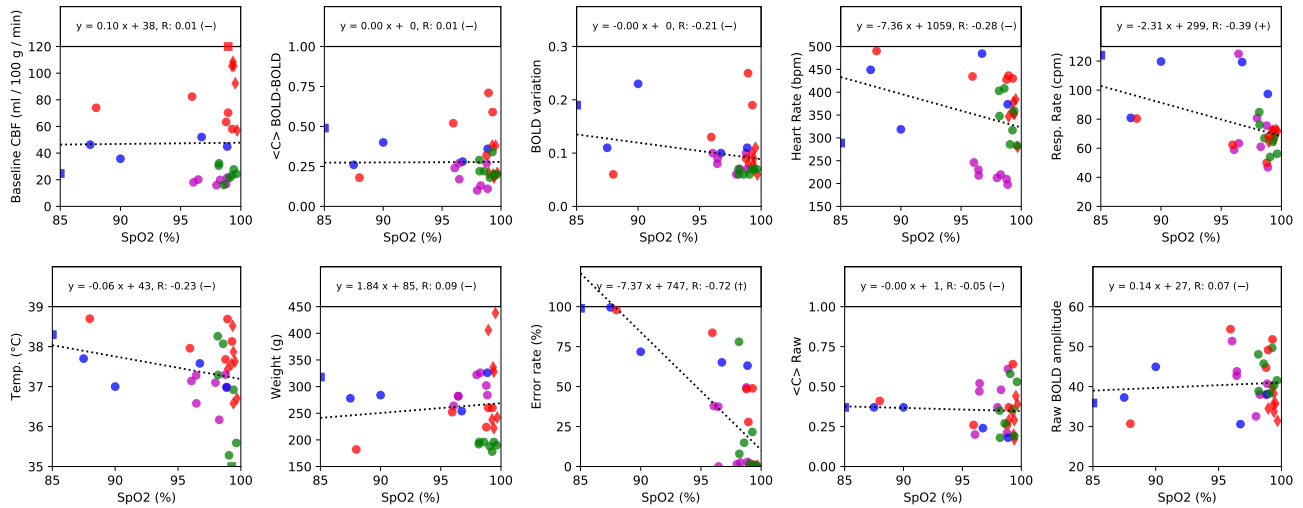


Figure I.23: Distributions — scatter plots — as a function of SpO2.

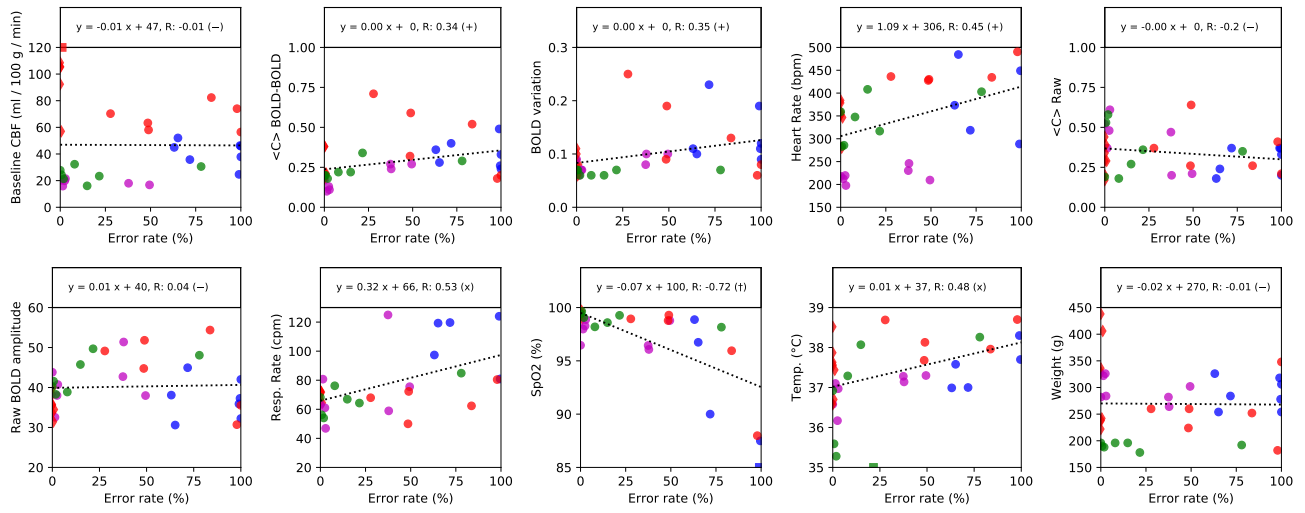


Figure I.24: Distributions — scatter plots — as a function of Error rate.

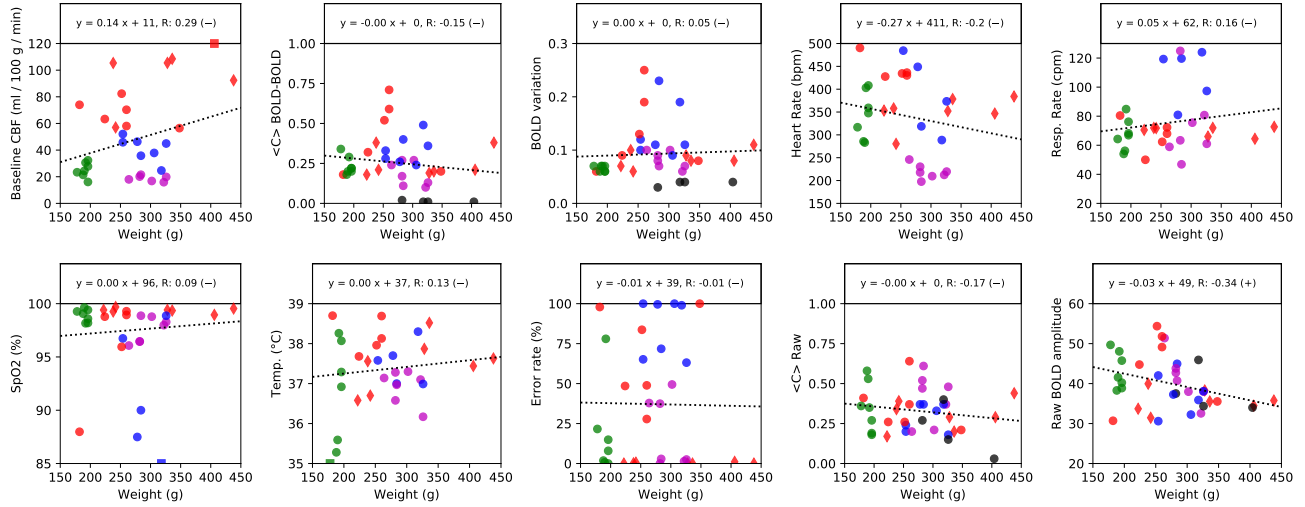


Figure I.25: Distributions — scatter plots — as a function of Weight.

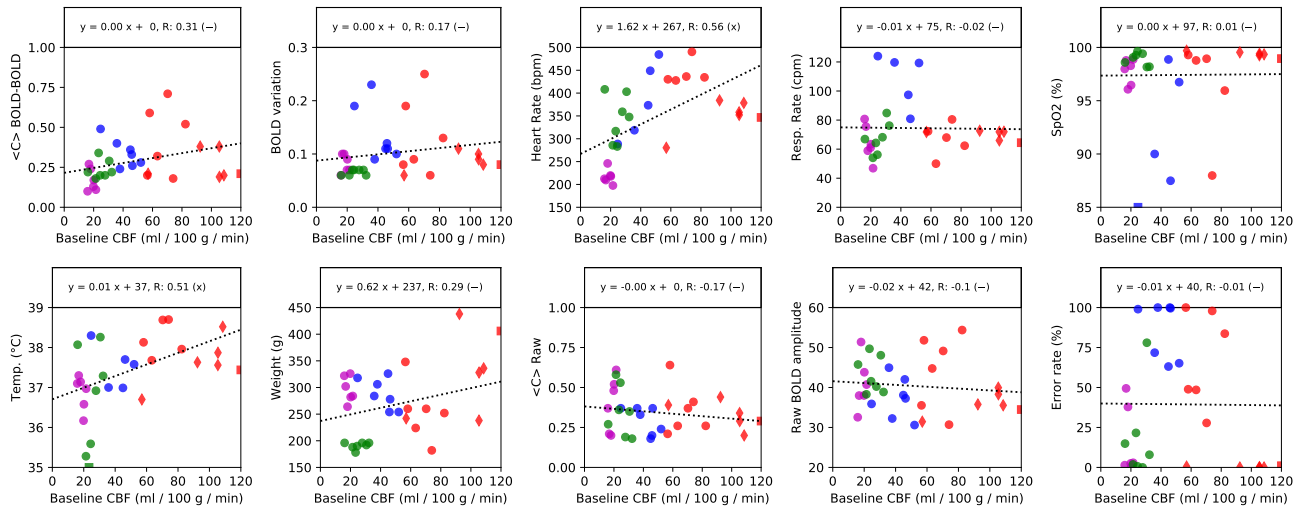


Figure I.26: Distributions — scatter plots — as a function of CBF.

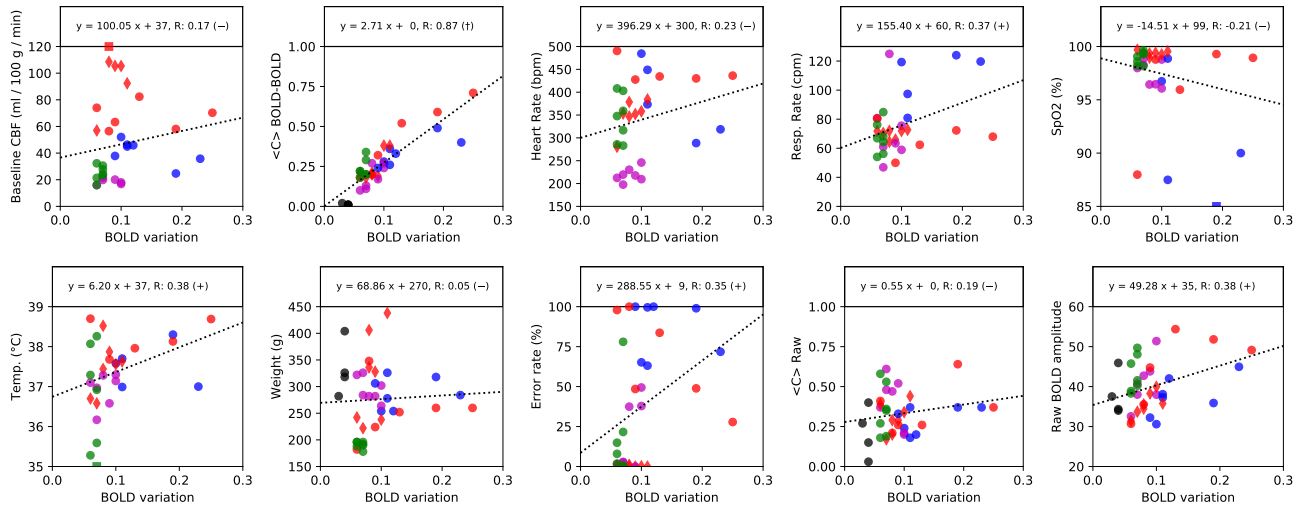


Figure I.27: Distributions — scatter plots — as a function of BOLD variations.

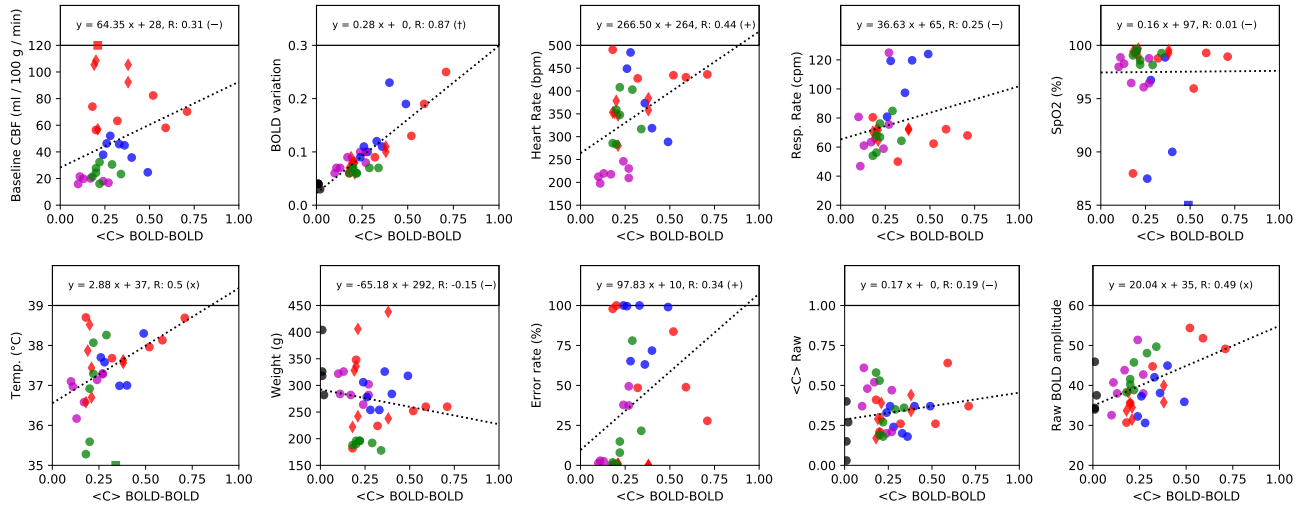


Figure I.28: Distributions — scatter plots — as a function of average correlations  $\langle C \rangle$ .

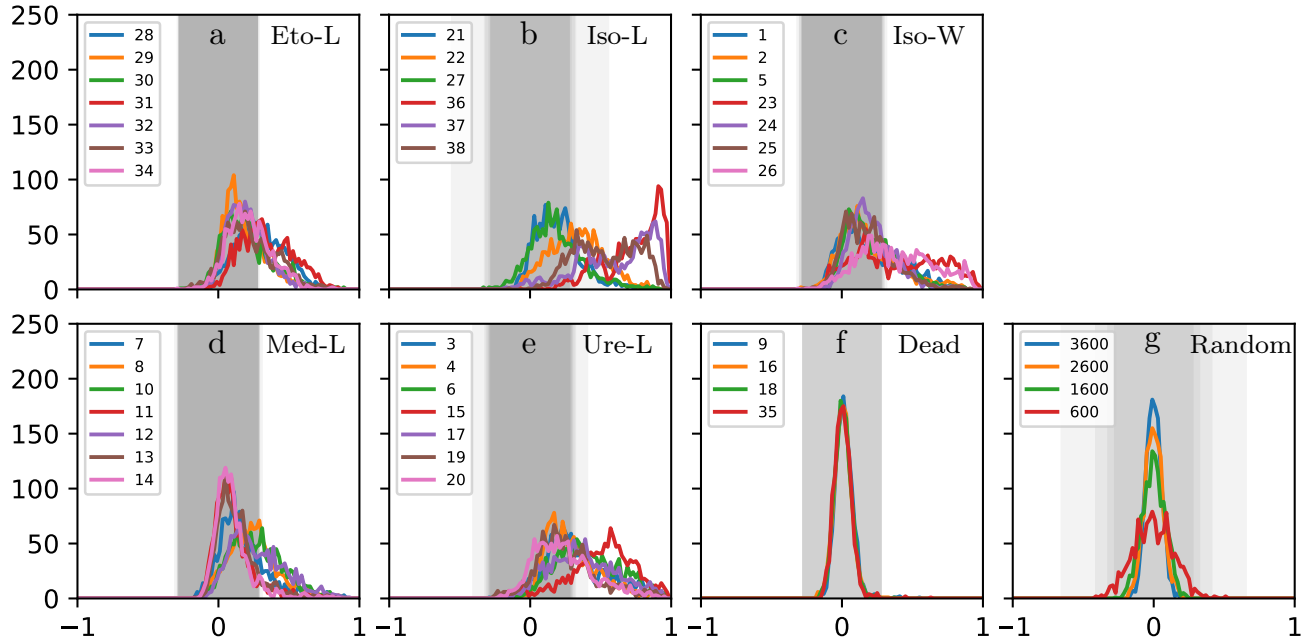


Figure I.29: Histograms of the BOLD-BOLD correlations per group in frequency band cD4. Histograms are estimated on 100 bins between  $[-1, 1]$ : a) – f) per anesthetic and for the Dead group with one color per animal; g) for simulated signals obtained with random values with the same number of areas and signal processing methods with different number of samples given in legend. The gray range represents the two-sided distribution of correlations below significance level given by the Bonferroni threshold.



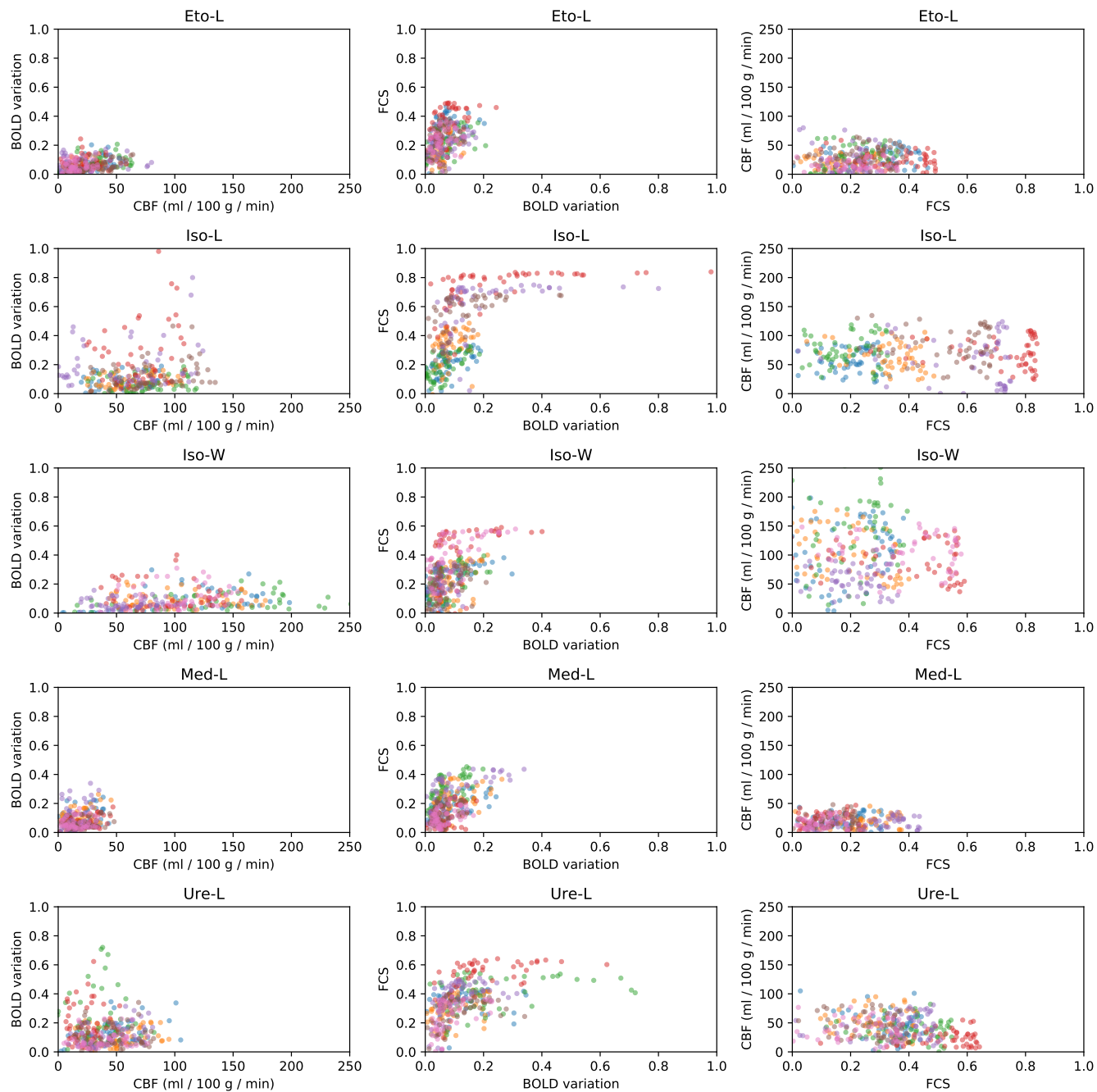


Figure I.30: Scatter plots per group between baseline CBF, BOLD variation and FCS. Dots with the same color belong to the same animal.

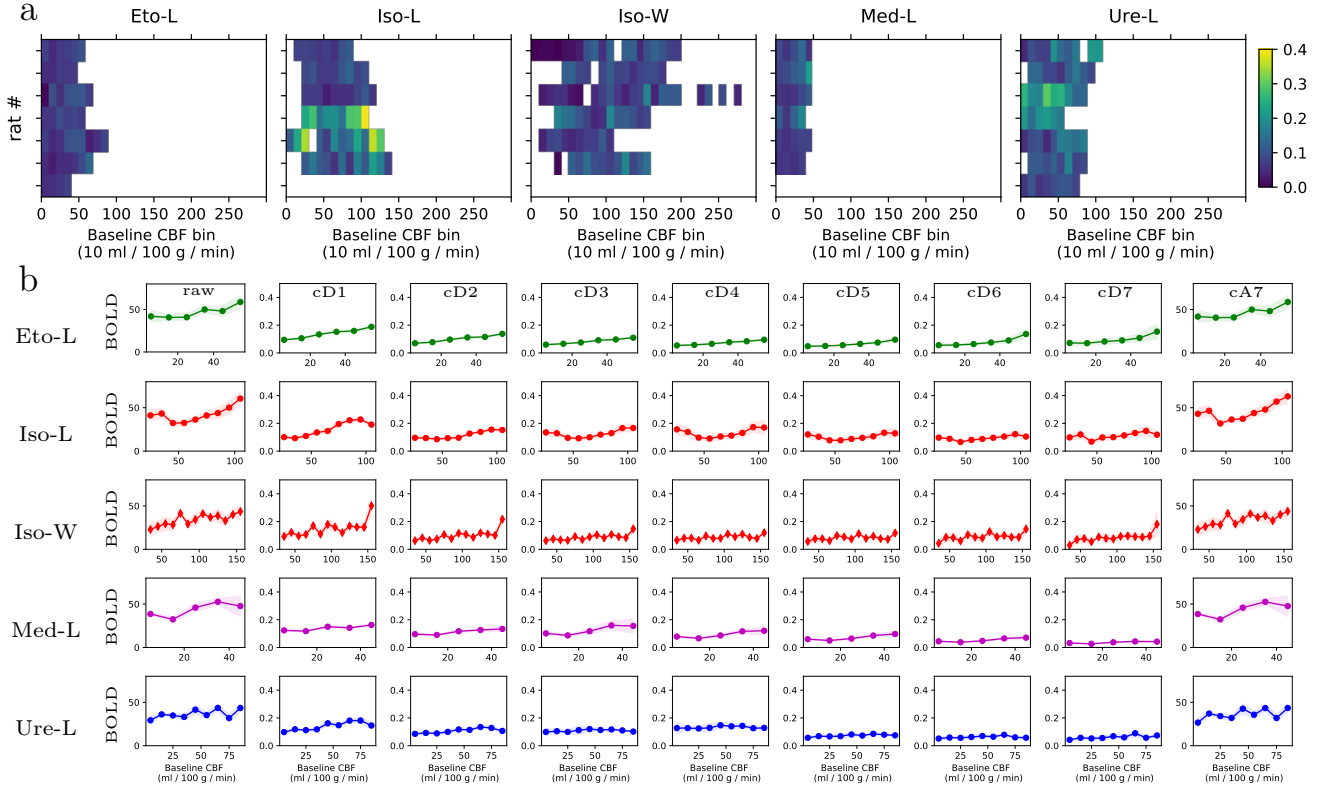


Figure I.31: Influence of population and frequency bands. a) BOLD variation as a function of CBF, for each anesthetized group. Each row represents one animal. BOLD variation is coded using the color scale on the right. b) BOLD amplitude for raw BOLD signal or variations versus CBF per frequency band and anesthetized group. BOLD variation in the cD4 band is presented in the main paper.

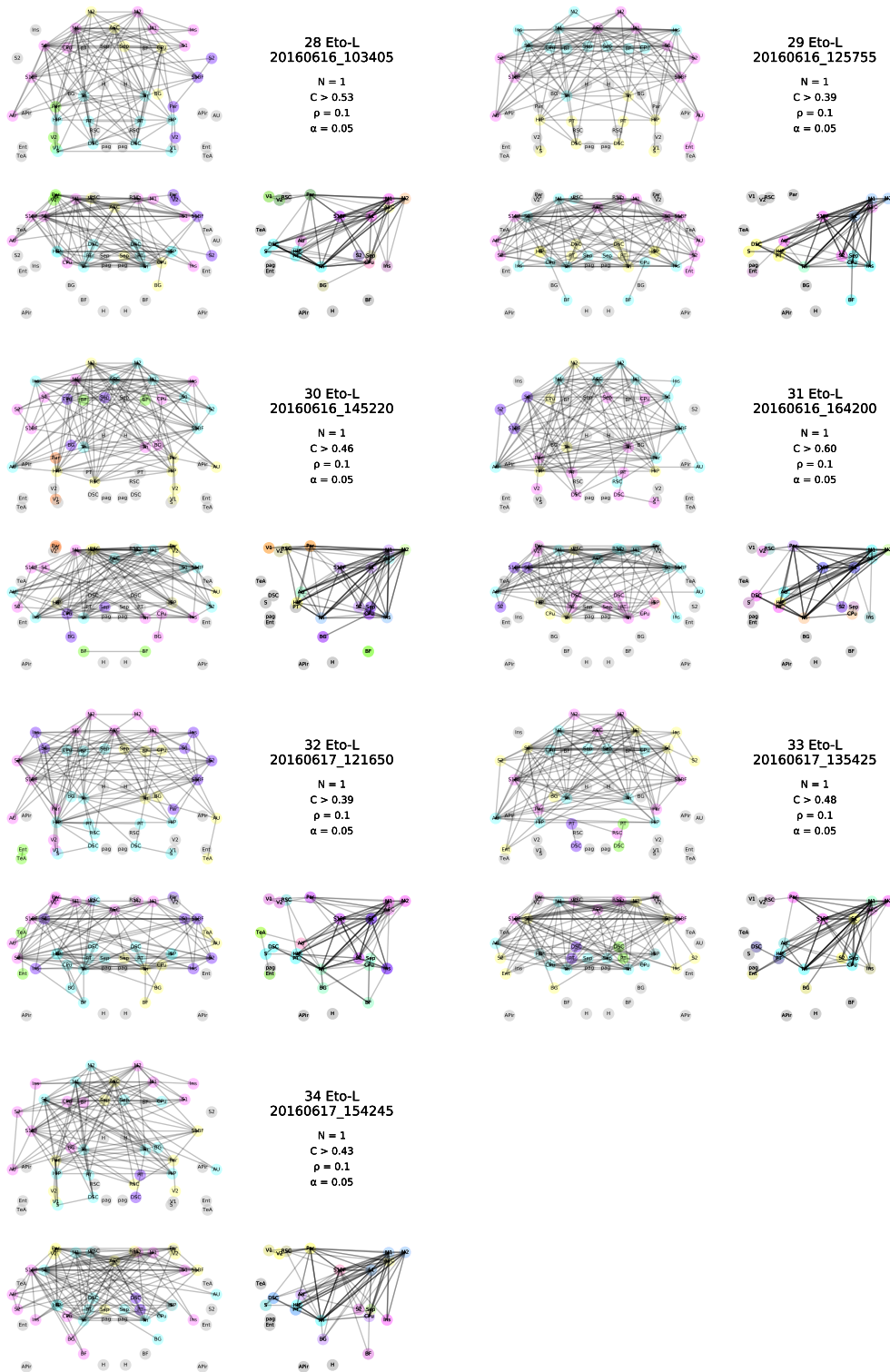


Figure I.32: Individual graph - Eto-L

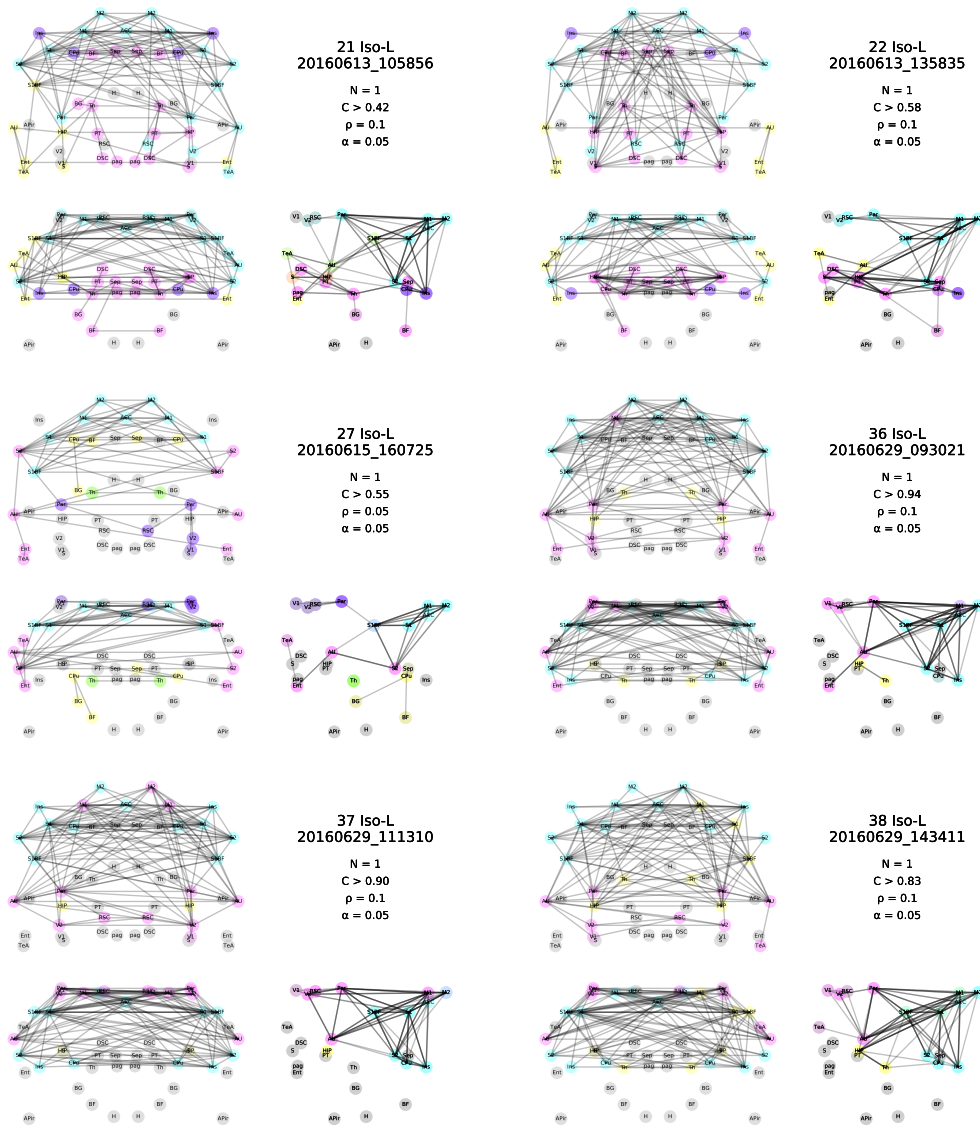


Figure I.33: Individual graph - Iso-L



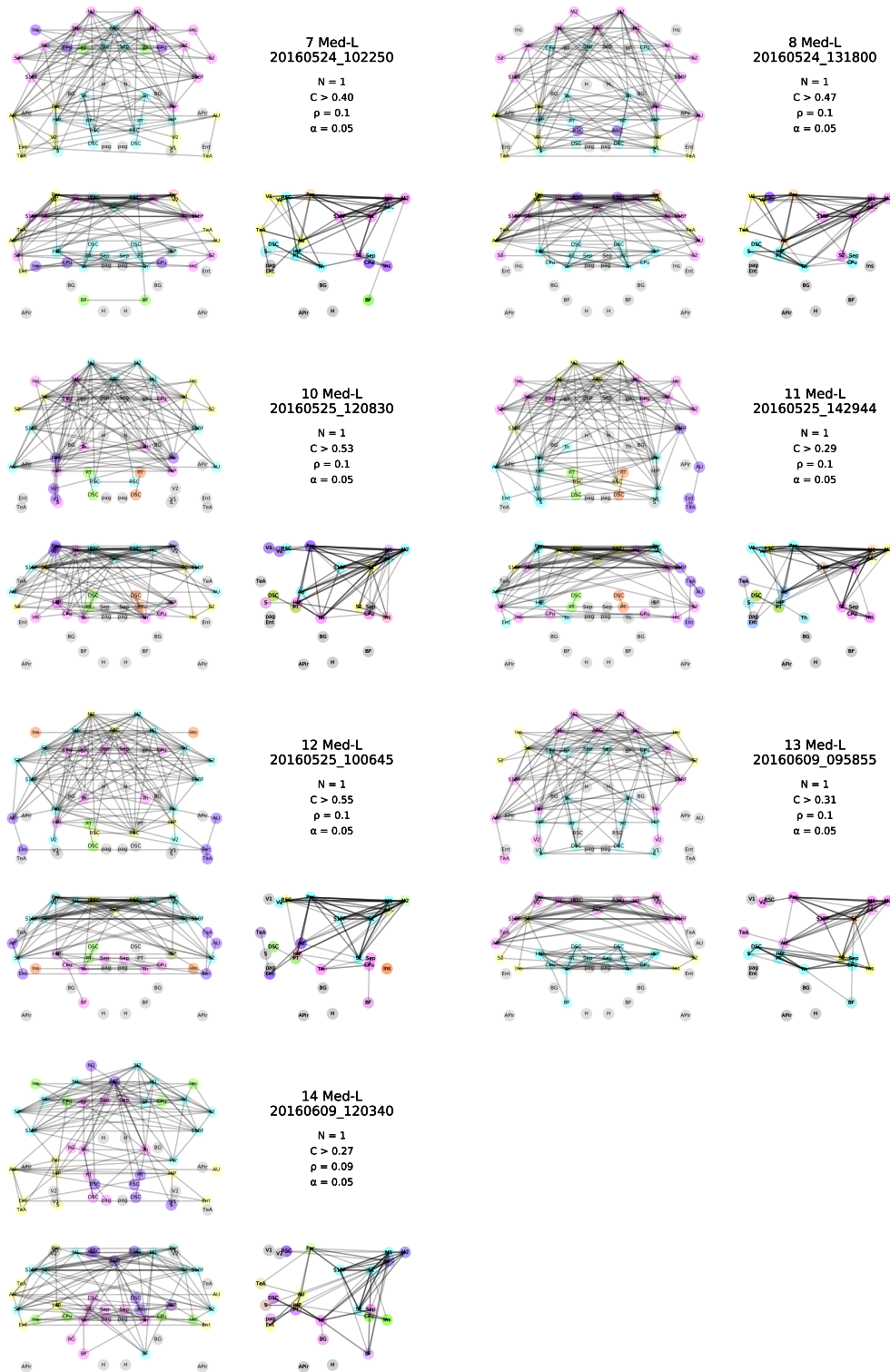


Figure I.35: Individual graph - Med-L



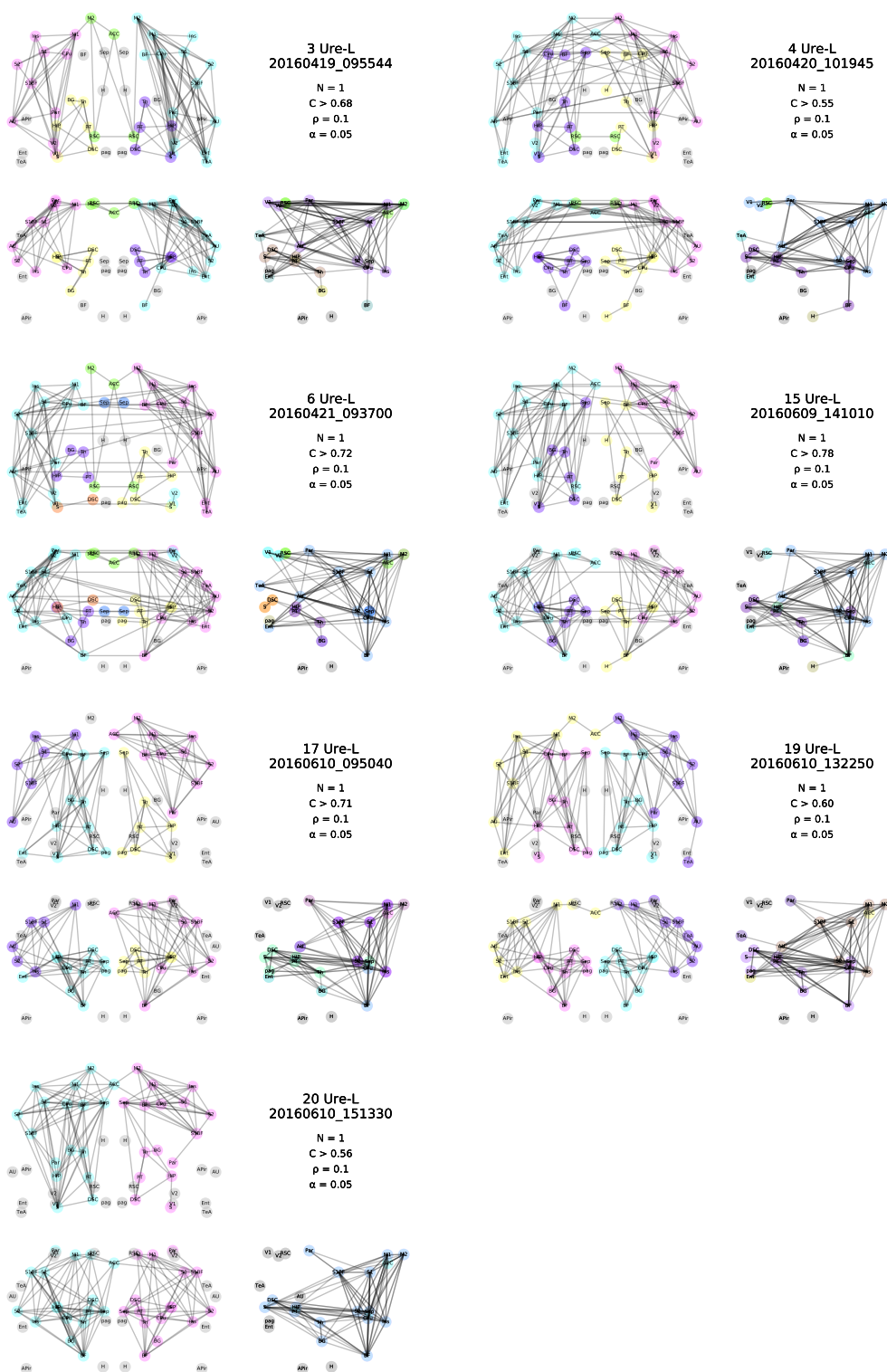


Figure I.36: Individual graph - Ure-L

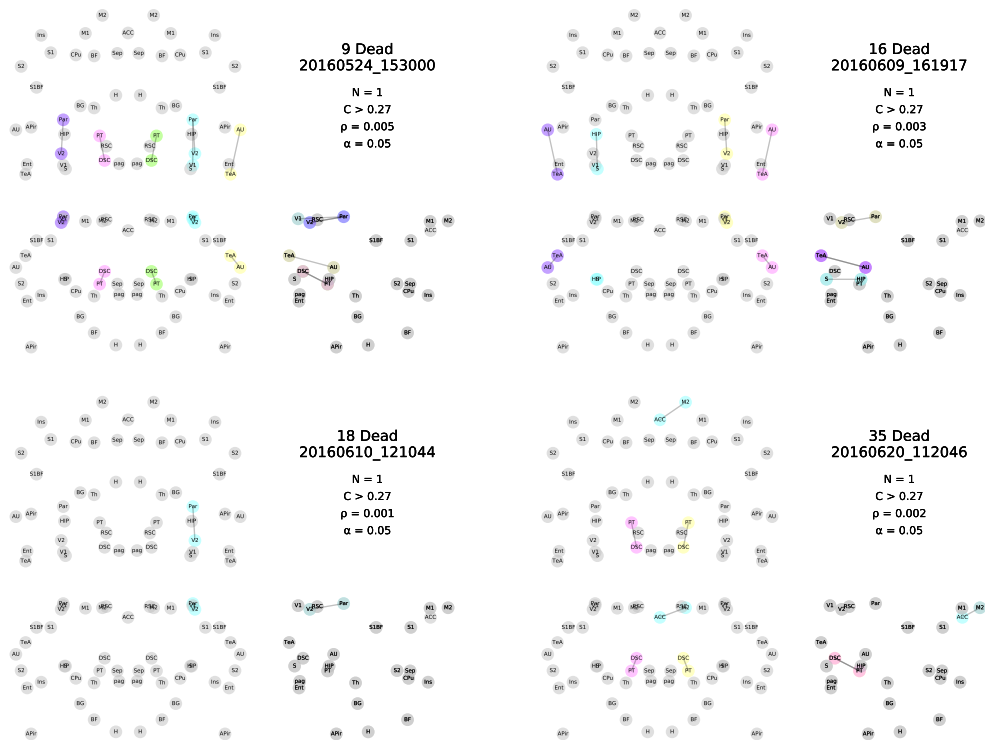


Figure I.37: Individual graph - Dead



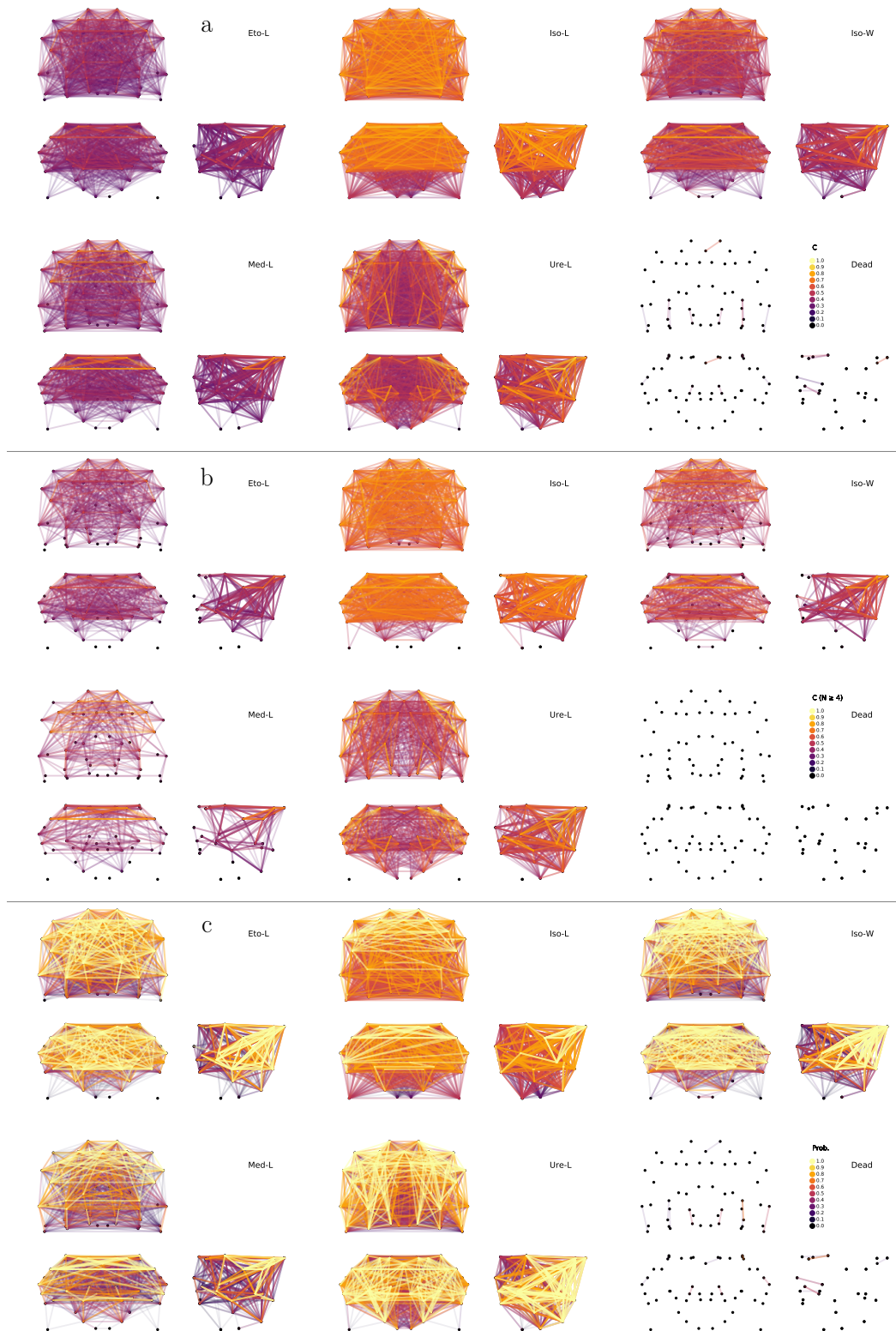


Figure I.38: Sorted Full Graph - Graphs with superimposed edges with the lowest on the background and the highest on the foreground. In these graphs, all available statistically significant edges are plotted for: a) average correlations; b) average correlations with at least 4 animals; c) probability of significant connections. Colors represent weights truncated at one decimal.